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Biochemical and molecular characterization of taurine:pyruvate aminotransferase from the anaerobe *Bilophila wadsworthia*

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Bilophila wadsworthia RZATAU is a Gram-negative bacterium which converts the sulfonate taurine (2-aminoethanesulfonate) to ammonia, acetate and sulfide in an anaerobic respiration. Taurine:pyruvate aminotransferase (Tpa) catalyses the initial metabolic reaction yielding alanine and sulfoacetaldehyde. We purified Tpa 72-fold to apparent homogeneity with an overall yield of 89%. The purified enzyme did not require addition of pyridoxal 5′-phosphate, but highly active enzyme was only obtained by addition of pyridoxal 5′-phosphate to all buffers during purification. SDS/PAGE revealed a single protein band with a molecular mass of 51 kDa. The apparent molecular mass of the native enzyme was 197 kDa as determined by gel filtration, which indicates a homotetrameric structure. The kinetic constants for taurine were: $K_m = 7.1$ mM, $V_{max} = 1.20$ nmol·s^{-1}, and for pyruvate: $K_m = 0.82$ mM, $V_{max} = 0.17$ nmol·s^{-1}. The purified enzyme was able to transaminate hypotaurine (2-aminosulfinate), taurine, β-alanine and with low activity cysteine and 3-aminopropanesulfonate. In addition to pyruvate, 2-ketobutyrate and oxaloacetate were utilized as amino group acceptors. We have sequenced the encoding gene (*tpa*). It encoded a 50-kDa peptide, which revealed 33% identity to diaminopelargonate aminotransferase from *Bacillus subtilis*.

Keywords: aminotransferase; *Bilophila wadsworthia*; taurine; anaerobic metabolism; pyridoxal 5′-phosphate.

Taurine (2-aminoethanesulfonate) is one of the most abundant organic low-molecular-mass solutes in many animals, including mammals [1]. Corresponding to this widespread occurrence, bacteria have been discovered which utilize this compound as carbon, nitrogen or sulfur sources under oxic or anoxic conditions [2]. Three types of attack on the taurine molecule are known, oxygenation [3], oxidation [4] and transamination (Fig. 1), which seems to be the most widespread in aerobic and anaerobic microorganisms. Two aminotransferases involved in aerobic dissimilation of taurine have been described: taurine:2-oxoglutarate aminotransferase in *Achromobacter superficialis* [5] and taurine:pyruvate aminotransferase in *Pseudomonas aeruginosa* [6]. Whereas the taurine:2-oxoglutarate aminotransferase has been examined in some detail [7], the taurine:pyruvate transaminase has not [6]; one enzyme with this tentative name has been reclassified as ω-amino-acid aminotransferase [8], and studied in detail. An inducible taurine:pyruvate aminotransferase is involved in assimilation of taurine sulfur during anaerobic growth of *Clostridium pasteurianum* C1 [9], but it has not been examined in detail. Furthermore, participation of taurine:pyruvate aminotransferase in anaerobic dissimilation of taurine was demonstrated in *Bilophila wadsworthia* RZATAU [10], in *Paracoccus pantotrophus* NKNCYSA [11], and in *Desulfonispora thiosulfatigenes* [12].

B. wadsworthia is a strictly anaerobic, Gram-negative bacterium [13], which can utilize taurine or other sulfonates as electron acceptor with formate as electron donor [10]. The initial metabolic reaction is a pyruvate-dependent transamination of taurine to sulfoacetaldehyde and alanine [10] (Fig. 1), and the literature shows no purification of a transaminase from the anaerobic degradative pathway for a sulfonate. So we chose to purify the taurine:pyruvate aminotransferase from *B. wadsworthia* RZATAU and to investigate its biochemical and molecular properties.

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Abbreviations: Tpa, taurine:pyruvate aminotransferase; *dsrAB*, genes encoding α and β subunit of dissimilatory sulfite reductase.

Enzymes: taurine:pyruvate aminotransferase (EC 2.6.1.-); taurine:2-oxoglutarate aminotransferase (EC 2.6.1.55); ω-amino acid:pyruvate aminotransferase (EC 2.6.1.18); diaminopelargonate aminotransferase (EC 2.6.1.62), acetylornithine aminotransferase (EC 2.6.1.11); alcohol dehydrogenase GbsB (EC 1.1.1.1); NAD-dependent methanol dehydrogenase (EC 1.1.1.244); alcohol dehydrogenase ADH4 (EC 1.1.1.1).

Note: the novel nucleotide sequence data published here have been submitted to the GenBank sequence databank and are available under accession number AF269146.

MATERIALS AND METHODS

Organism, growth and preparation of cell-free extracts

B. wadsworthia RZATAU (DSM 11045) was routinely grown in batch culture (0.1, 1 or 10 L) in an anoxic fresh-water mineral-salts medium containing 12 mM taurine and 80 mM formate [10]. Cells were also grown with 12 mM taurine with 25 mM pyruvate, 12 mM cysteate (2-amino-3-sulfopropionate) with 80 mM formate, 12 mM isethionate (2-hydroxyethanesulfonate) with 80 mM formate, 10 mM thiosulfate with 20 mM DL-lactate, or 30 mM pyruvate.

Bacteria from 10-L cultures were harvested at the end of exponential growth in a Pellicon filtration system (Millipore, Neu-Isenburg, Germany), centrifuged (13 000 ***g***, 40 min, 4 °C) and washed twice in Mops buffer (50 mM Mops/KOH, pH 6.5).

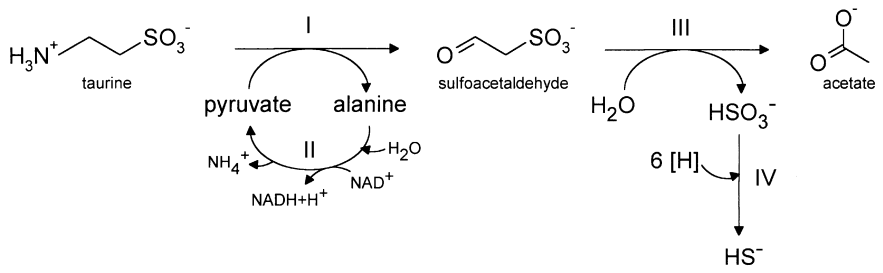

Fig. 1. Degradative pathway of taurine in *B. wadsworthia*. Taurine is degraded to the end products ammonia, acetate and sulfide by four metabolic enzymes: Tpa (I), alanine dehydrogenase (II), putative sulfoacetaldehyde sulfolyase (III), and dissimilatory sulfite reductase (IV) [10]. The initial step (I) is a pyruvate-dependent transamination of taurine to sulfoacetaldehyde and alanine. Oxidative deamination of alanine to pyruvate and ammonia is catalysed by alanine dehydrogenase [22]. Desulfonation of sulfoacetaldehyde in a presumably thiamine pyrophosphate-dependent reaction (III) yields acetate and putatively sulfite. The sulfite is reduced to sulfide by a dissimilatory sulfite reductase (IV) [36] with reduction equivalents derived mainly from formate oxidation and from oxidation of the taurine carbon.

The cell pellets were stored frozen for several weeks at −20 °C without significant loss of activity. Frozen cells were thawed, resuspended to 0.5 g (wet weight) per ml in 50 mM Mops (pH 6.5) containing 10 μM pyridoxal 5′-phosphate and ruptured by four passages through a chilled French pressure cell followed by centrifugation to remove intact cells and debris (30 000 ***g***, 20 min, 4 °C). After an ultracentrifugation step (160 000 ***g***, 40 min, 4 °C) nucleic acids were removed by precipitation with 2% (v/v) streptomycin sulfate as described elsewhere [14].

Cells (200 mL or 100 mL) of a 1-L culture grown with taurine plus formate were harvested during different phases of growth and washed twice in 0.1 Tris/HCl, pH 7.5. The resuspended cells (0.25 g wet weight per mL) were disrupted by sonification and intact cells were removed by centrifugation (30 000 ***g***, 5 min, 22 °C).

The influence of buffers and additives on the stability of Tpa activity was studied in cells ruptured by sonification in different buffers (each 50 mM): piperazine, pH 6.0; Bistris, pH 6.0; Mes, pH 6.0, 6.5; Mops, pH 6.5–7.5; Hepes, pH 7, 7.5; K-phosphate, pH 7.0, 7.5; triethanolamine, pH 7.5, 8.0; and Tris/HCl, pH 7.5–8.5. Further, 20% glycerol, 1 mM phenylmethanesulfonyl chloride, 5 mM EDTA, 1 mM dithiothreitol, 0.1% 2-mercaptoethanol or 0.1 mM pyridoxal 5′-phosphate were added to 50 mM Tris/HCl buffer, pH 7.5. The cell-free extracts were stored on ice or at 22 °C and Tpa activity was determined at intervals.

Enzyme assays and analytical methods

Tpa was routinely assayed as the formation of alanine from taurine and pyruvate at 35 °C. The assay mixture contained, in 1 mL total volume, 100 mM Tris/HCl, pH 9.0, 5 mM taurine, 5 mM pyruvate, 0.1 mM pyridoxal 5′-phosphate. The reaction was started by the addition of enzyme. After 2–5 min the reaction was stopped by the addition of 50 μL of a hydrazine (10 M)/EDTA (14 mM) solution. Enzymes were inactivated by heating at 95 °C for 10 min. The concentration of alanine in the 1-mL reaction mixture was measured enzymatically with alanine dehydrogenase [15].

The kinetic constants of the Tpa were measured using the discontinuous alanine dehydrogenase test as described above by varying the concentrations of taurine or hypotaurine at a pyruvate concentration of 10 mM or by varying the concentration of pyruvate at a taurine concentration of 10 mM. The data for the enzyme kinetics were fitted to Michaelis–Menten kinetics and K_m and V_{max} values were calculated by plotting v against v/c (Eadie–Hofstee plot).

The temperature dependence of the enzyme was determined by preincubating the reaction mixture for 10 min at a temperature ranging from 15 to 60 °C and subsequently starting the reaction by addition of enzyme. The pH dependence of the Tpa activity was tested in a buffer system consisting of 100 mM Aces, 52 mM Tris and 52 mM ethanolamine [16] at pH 6–11. The alanine concentration was measured in 100 μL aliquots.

Consumption of taurine and formation of alanine were confirmed by HPLC analysis after derivatization with 2,4-dinitrofluorobenzene [17]. Reverse-phase chromatography with diode array detection was performed as described elsewhere [18]. Sulfoacetaldehyde was detected qualitatively by the aldehyde reagent *o*-aminobenzaldehyde [19] or quantitatively after derivatization with 2-(diphenylacetyl)indane-1,3-dione 1-hydrazone [20] and UV detection by HPLC. Protein concentrations were determined by the method of Bradford [21] with bovine serum albumin as standard.

The reverse reaction of the Tpa (i.e. formation of taurine and pyruvate from sulfoacetaldehyde and alanine) was measured in the presence of about 10 mM bisulfite adduct of sulfoacetaldehyde, 5 mM L-alanine, 0.1 mM pyridoxal 5′-phosphate and purified enzyme (9 μg) in 100 mM Tris/HCl, pH 9.0. Taurine and alanine concentrations were determined by HPLC.

Ultraviolet/visible spectra of the enzyme at different pH values (50 mM acetate, pH 4.8; 50 mM Mops, pH 6.5; 50 mM Tris/HCl, pH 8.0) were recorded on a Uvikon 922 spectrophotometer (Kontron). The enzyme was reduced with 86 nmol borohydride in Mops buffer. Fluorescence spectra of Tpa in 0.1 M potassium phosphate, pH 7.0 were recorded on a F-2000 fluorimeter (Hitachi).

The sources of the chemicals and gases (N_2/CO_2) used were given elsewhere [22]. Sulfoacetaldehyde was synthesized as bisulfite adduct [23] and its identity confirmed by NMR and IR (K. Denger, Universitat Konstanz, Konstanz, Germany, personal communication).

Purification of Tpa

All chromatographic steps were performed at room temperature with a Pharmacia FPLC system (DEAE-Sepharose)

or with Beckman HPLC apparatus (Mono Q, Phenyl-Superose, Superose 12). Pyridoxal 5′-phosphate (10 μM) was added to all buffers during the purification procedure. Absorption of column effluents was monitored at 280 nm. Where required between purification steps, fractions were combined and concentrated by membrane filtration (30-kDa cut-off, Intersep, Witten, Germany) in a stirring cell (Amicon) or by centrifugal ultrafiltration (10-kDa cut-off, Intersep). Purified protein was routinely stored at −20 °C. Tpa was purified in a three-step protocol.

Step 1. The Mono Q (HR 10/10; Pharmacia) anion-exchange column was equilibrated with 20 mM Mops, pH 6.5 at 2 mL·min^{-1}. Crude extract diluted to 20 mM Mops was loaded onto the column and 5 mL fractions were collected. After washing with 60 mL of start buffer, a gradient from 0 to 0.05 M Na_2SO_4 was applied over the next 8 mL followed by a gradient from 0.05 to 0.2 M over 160 mL. The remaining proteins were eluted with an increasing gradient from 0.2 to 1 M Na_2SO_4 over 20 mL. Tpa eluted at about 60 mM Na_2SO_4 in yellow-coloured fractions.

Step 2. The Phenyl-Superose hydrophobic interaction column (HR 5/5; Pharmacia) was equilibrated with 20 mM Mops, pH 6.5 containing 1.2 M $(NH_4)_2SO_4$ at a flow rate of 0.2 mL·min^{-1}. Protein from step 1 was brought to 1.2 M $(NH_4)_2SO_4$ by addition of 3 M $(NH_4)_2SO_4$ and loaded onto the column. After washing for 5 mL, the concentration of $(NH_4)_2SO_4$ was decreased from 1.2 to 0 M over 15 mL elution volume. Fractions of 1 mL were collected. Tpa eluted at about 0.7 M $(NH_4)_2SO_4$ in slightly yellow-coloured fractions.

Step 3. Protein from step 2 was loaded onto a gel filtration column (Superose 12, HR 10/30; Pharmacia) equilibrated with 50 mM Mops (pH 6.5) containing 150 mM Na_2SO_4. The flow rate was 0.4 mL·min^{-1} and 0.5 mL fractions were collected. The molecular masses of the proteins used to calibrate the column were ferritin (440 kDa), catalase (232 kDa), aldolase (158 kDa), albumin (67 kDa) and ovalbumin (43 kDa).

Step 1 was modified for purification of larger amounts of Tpa. Crude extract was applied to a DEAE-Sepharose anion-exchange column (80 mL) [22] instead of the Mono Q column. Tpa eluted at about 0.1 M NaCl.

Gel electrophoresis and N-terminal sequence analysis

Proteins were separated by SDS/PAGE (12%) [24] and stained with Coomassie Brilliant Blue R-250 [25]. The homogeneous protein in an SDS/PAGE was blotted onto a poly(vinylidene difluoride) membrane for N-terminal sequencing. The blotted protein was stained with Coomassie R-250, cut out and subjected to Edman degradation in an Applied Biosystems 477 A gas-phase system. The protein was separated on an SDS/PAGE and stained [26] prior to determination of internal amino-acid sequences. The band was cut out, digested with the protease LysC and the resulting peptides were separated by reverse-phase HPLC. Two peptide fractions (K-23, K-13) were sequenced (TopLab GmbH, Munich, Germany).

DNA sequencing and analysis

Degenerate oligonucleotide primers TpaNTF, TpaK23R and TpaK13R (5′-GTSGCCCTSGACAAGAAGTACG-3′, 5′-YTC-GTTRGCTTCGGARCCGGAG-3′ and 5′-CTTGCCRC-GSACGTCGCCGATGATSGGGTG-3′) were derived from the N-terminal amino-acid sequence VALDKKYV and from the internal peptides of the Tpa SGSEANE (K-23) and HPIIGDVRGK (K-13). PCR was performed with washed and fivefold concentrated cells of *B. wadsworthia* RZATAU or total DNA [27] using the MBI *Taq* polymerase system (MBI Fermentas) but with a modified reaction buffer [22]. The primer pair TpaNTF-TpaK23R amplified a 300-bp fragment, whereas the primer pair TpaNTF-TpaK13R amplified a 1.1-kb fragment, which was sequenced from both strands by cycle sequencing using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit and an ABI 377 DNA sequencer (GATC GmbH).

We applied adaptor-ligated PCR to obtain the complete sequence of the *tpa* gene. Total DNA from *B. wadsworthia* was digested and ligated to an adaptor of known sequence (Universal Genome Walker™ Kit, Clontech). Nested PCR was performed with the Advantage Genomic Polymerase Mix (Clontech) and primer deduced from gene specific sequences in combination with adaptor primer. Amplification products were obtained containing the 5′ or the 3′ end of the *tpa* gene. Database searches were performed with BLAST [28].

RESULTS AND DISCUSSION

Activity and stability of Tpa in cell-free extracts of *B. wadsworthia*

Tpa activity was determined in cell-free extracts of *B. wadsworthia* RZATAU grown with different substrates. The specific activity was higher in pyruvate-grown cells (37 mkat·kg protein^{-1}; relative activity 100%) than in taurine/formate (17 mkat·kg protein^{-1}; 45%), isethionate/formate (30%) or taurine/pyruvate-grown cells (27%). Lower activities were detected in extracts of cells grown with thiosulfate/lactate (14%) or cysteate/formate (9%).

We compared Tpa activity in cell-free extracts from taurine/formate-grown cells during growth. Maximum activity in this experiment (5.8 mkat·kg protein^{-1}) was detected in extracts of cells harvested at a D_{580} of 0.53, which represented

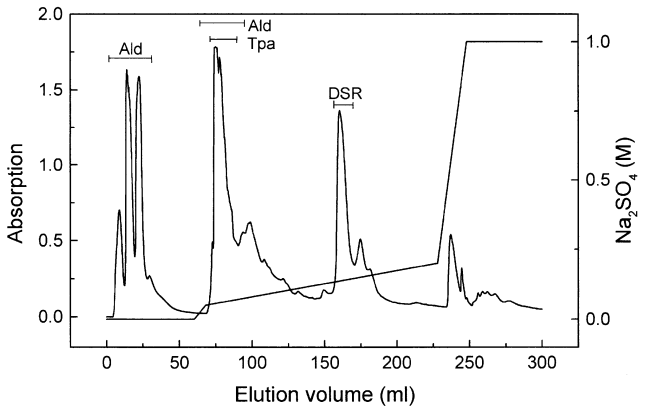

Fig. 2. Separation of enzymes involved in taurine degradation of *B. wadsworthia* RZATAU on an anion-exchange column. Crude extract was loaded in three portions onto the Mono Q column and eluted with a gradient of Na_2SO_4. Protein was monitored as absorption at 280 nm. Alanine dehydrogenase activity (Ald) was determined as the reduction of NAD^+ in the presence of 50 mM L-alanine, 1.5 mM NAD^+, 0.1 M CAPS, pH 10.0 [22]. Tpa activity (Tpa) was determined as described in Methods. Dissimilatory sulfite reductase (desulfoviridin) (DSR) was located due to its absorption at 630 nm.

Table 1. Purification of Tpa from *B. wadsworthia* RZATAU. Activity was measured as formation of alanine from taurine in a discontinous assay.

Purification step	Protein (mg)	Activity ($nmol \cdot s^{-1}$)	Specific activity ($mkat \cdot kg^{-1}$)	Yield (%)	Purification (-fold)
Crude extract	45.4	371	8.16	100	1.00
Mono Q	12.0	1630	135	439	16.6
Phenyl-Superose	0.94	442	470	119	57.6
Superose 12	0.56	330	589	88.9	72.2

0.12 mg protein·ml^{-1}, shortly before the end of the exponential growth phase. At the end of growth, the activity decreased, in 2 h to 50% (not shown). Therefore we harvested the cells at a D_{580} of 0.5–0.6.

During the initial purification experiments with Tpa we had significant losses (90%) of activity in Tris/HCl buffer (pH 7.5), so we tested the stability of enzyme activity in cell-free extracts after preincubation in the presence of additives and in different buffers. Addition of protease inhibitors, reducing agents or pyridoxal 5′-phosphate to Tris/HCl buffer, pH 7.5 had no significant effect, though the enzyme was more stable in the presence of 20% glycerol. Loss of activity was observed after preincubation in buffers at neutral to alkaline pH values (see Materials and methods) except for Hepes at pH 7, and to lower extent at pH 6 in piperazine and Bistris. However, Tpa activity remained constant during storage for 22 h at 4 °C or at 22 °C in the sulfonate buffers Mops and Mes in the pH range 6–7, which indicated a stabilizing effect of sulfonated buffers and weakly acidic pH. We chose to use Mops buffer, pH 6.5 for all purification steps.

Purification of Tpa

Tpa was purified 72-fold from the soluble fraction of crude extract from taurine/formate grown cells by a three-step purification involving anion exchange (Fig. 2), hydrophobic interaction and gel filtration chromatography (Table 1). After gel filtration, SDS/PAGE analysis showed one single, homogeneous protein band (Fig. 3). The addition of pyridoxal 5′-phosphate to all buffers was essential for purification of an active protein. Without this addition, the yield was only 30–50% after Mono Q, and 5% after hydrophobic interaction chromatography. During the Mono Q step, the presence of added pyridoxal 5′-phosphate increased the measured activity about fourfold, whereas with DEAE-Sepharose in place of Mono Q only a twofold increase was found.

The Tpa of *B. wadsworthia* RZATAU catalyses the initial metabolic step of anaerobic taurine degradation, i.e. pyruvate-dependent transamination of taurine to sulfoacetaldehyde and alanine (see below; Fig. 1). The specific activity of Tpa in crude extracts, e.g. 8.2 mkat·kg $protein^{-1}$ (Table 1) is higher than the activity calculated to be required for growth (2.7 mkat·kg $protein^{-1}$ [10], and it is the only taurine:pyruvate aminotransferase found in crude extract (Fig. 2). So we are convinced that we have purified the correct enzyme, which, given a 72-fold purification (Table 1) represents about 1.4% of soluble protein. The yield of 89% (Table 1) could only be achieved with pyridoxal 5′-phosphate in the buffers during the purification. Reactivation of the enzyme by preincubation with the cofactor, used to obtain taurine:2-oxoglutarate aminotransferase from *A. superficialis* [29], gave much lower yields. The pyridoxal 5′-phosphate in the elution buffers bound to the anion exchange columns, where it coeluted with Tpa on Mono Q, but was partially separated on DEAE-Sepharose; this may explain the lower specific activity observed after DEAE-Sepharose.

Molecular properties

The molecular mass of the denatured protein determined by SDS/PAGE was 51 kDa (Fig. 3). The enzyme eluted from gel-filtration columns under nondenaturing conditions with an apparent molecular mass of 197 kDa, but minor peaks corresponding to sizes of 95 kDa and 350 kDa were also observed. All of these fractions contained active enzyme and showed a 51-kDa band in SDS/PAGE. N-terminal sequencing of the purified protein yielded a unique sequence, so we presume a homotetrameric structure and the presence of other aggregates.

The tetrameric structure resembles that of ω-amino acid: pyruvate aminotransferase from *P. putida*, with its native molecular mass of about 172 kDa [30] and a 49-kDa subunit [31]. We believe the other structures observed on gel filtration to be artifacts. The formation of large aggregates (350 kDa)

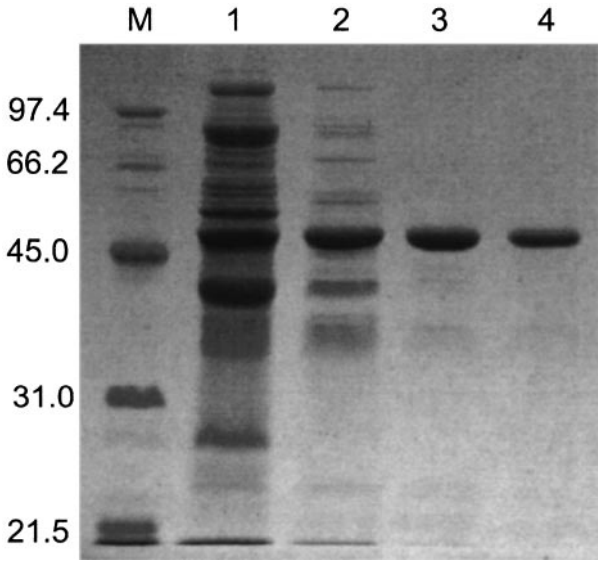

Fig. 3. Purification of Tpa monitored by SDS/PAGE. Proteins in samples from different stages of the purification were separated on a 12% SDS polyacrylamide gel and subsequently stained with Coomassie Brilliant Blue. Lanes: M, molecular mass standards (masses are given in kDa); 1, crude extract (30 μg of protein); 2, Tpa from Mono Q chromatography (10 μg); 3, active fraction from Phenyl sepharose (4 μg); 4, purified Tpa after gel filtration on Superose 12 (2 μg).

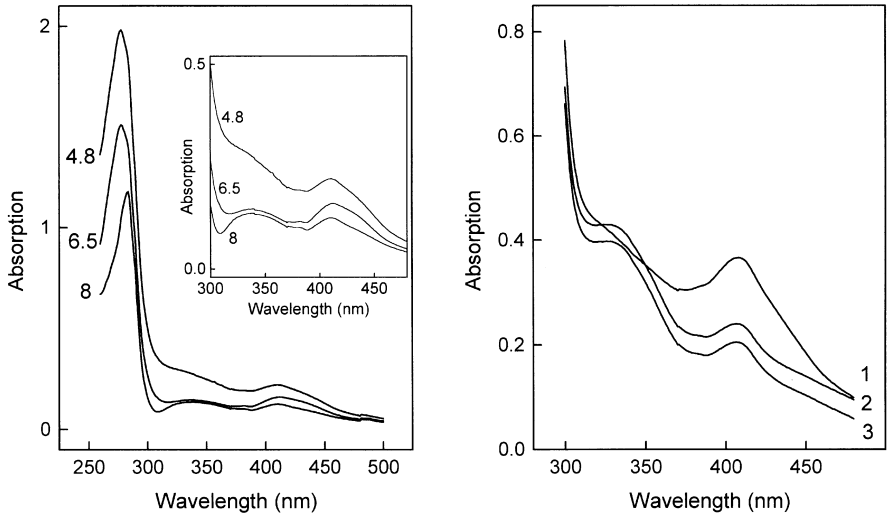

Fig. 4. UV/Vis spectrum of Tpa at different pH values and after reduction with borohydride. (A) Absorption was monitored in acetate buffer, pH 4.8; Mops buffer, pH 6.5, and Tris/HCl buffer, pH 8.0. (B) At pH 6.5 the enzyme (1) was reduced with 86 nmol (2) and 172 nmol borohydride (3).

depended on storage conditions; freezing (− 20 °C) or freezing rapidly in liquid nitrogen (with storage at −20 °C) increased this form, the presence of glycerol decreased it. The apparent dimer (95 kDa) was observed only in enzyme preparations stored at 4 °C.

Purified Tpa showed absorption maxima at 278 nm and 411 nm and a shoulder at 330 nm at pH 6.5 (Fig. 4A). The absorption was influenced by pH. After reduction with borohydride, absorption at 330 nm increased (Fig. 4B). After excitation at 330 nm, the enzyme emitted fluorescence with a maximum at 396 nm. Excitation at 410 nm yielded low emission of fluorescence between 500 and 600 nm. We attribute these properties largely to the bound pyridoxal 5′-phosphate.

N-terminal amino-acid sequencing of the purified Tpa yielded the following sequence: TYDKAELVALDKKY-VWHHLTQHKNFEP. In addition, the enzyme was digested with the protease LysC and the amino acids of two of the resulting peptides were determined: (K)MPGMSRVYLSN-SGSEANEK (K-23); (K)HPIIGDVRGK (K-13). The first 20 N-terminal amino acids of the protein revealed 50% sequence identity to the N-terminal amino-acid sequences of the diaminopelargonate-aminotransferase from *Saccharomyces cerevisiae* [32].

Catalytic properties

Tpa catalysed the transamination of taurine with pyruvate as amino-group acceptor to sulfoacetaldehyde and alanine (Fig. 1). Enzyme activity was determined routinely in a discontinuous assay as the formation of alanine, which was measured enzymatically by commercial alanine dehydrogenase. The assay was linear up to 5 min of transamination and a linear relationship between protein concentration (from 0.3 μg to 7 μg protein) and amount of alanine formed was observed. No difference in activity was detected in the absence or presence of pyridoxal 5′-phosphate in the assay system. Alanine was identified by cochromatography (HPLC) after derivatization. Formation of an aldehyde as product of the transamination was indicated by the reaction with *o*-aminobenzaldehyde [19] and identification as sulfoacetaldehyde was performed by HPLC analysis and cochromatography with authentic material. Confirmation of the reversibility of the reaction came from formation of taurine from alanine and sulfoacetaldehyde (Fig. 5). Disappearance of 1.8 mM alanine was concomitant with formation of 1.9 mM taurine in 15 min, which confirmed the unit stoichiometry of the reaction. No taurine formation was observed in the absence of alanine or in the absence of enzyme (Fig. 5).

The pH optimum observed for the Tpa was 9.0. The enzyme displayed a broad temperature optimum between 20 and 40 °C with maximum activity at 35 °C. The substrate specificity of Tpa (Table 2) and some kinetic constants were investigated. The apparent K_m values for taurine, pyruvate and hypotaurine were determined to be 7.1, 0.82 and 8.1 mM, respectively; the corresponding V_{max} values were 1.2, 0.17 and 1.3 nmol·s^{-1}. The range of amino-group donors was determined in the presence of pyruvate as acceptor as the amount of alanine formed (Table 2). In addition to pyruvate, the aminotransferase utilized as amino-group acceptor with taurine as donor 2-keto-butyrate yielding 2-aminobutyrate, and oxaloacetate, whose product could not be identified; it was not aspartate (Table 2).

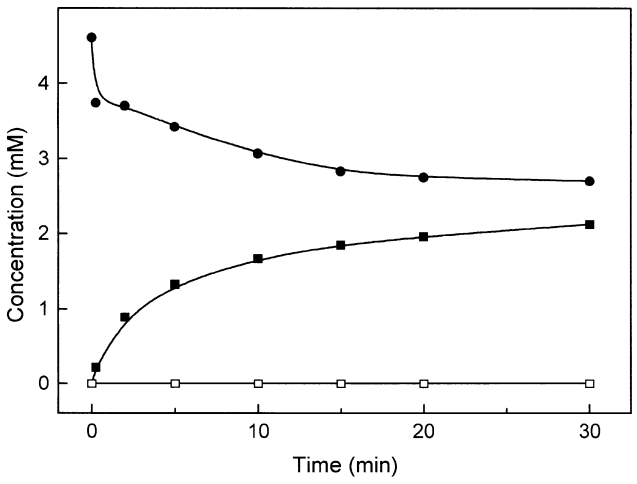

Fig. 5. Formation of taurine from sulfoacetaldehyde and alanine in the reverse reaction of Tpa. The reaction was performed with purified Tpa (9 μg protein) in 100 mM Tris/HCl, pH 9.0 containing about 10 mM bisulfite adduct of sulfoacetaldehyde, 5 mM alanine and 0.1 mM pyridoxal 5′-phosphate at 35 °C. Samples were taken in intervals for HPLC determination of alanine (●) and taurine (■). No taurine (□) was detected in a control experiment without alanine.

Table 2. Substrates of the Tpa. Activity (rates) for amino-group donors was determined enzymatically as the formation of alanine with alanine dehydrogenase in the presence of 2 mM pyruvate, 0.1 mM pyridoxal 5′-phosphate in 50 mM Tris/HCl, pH 9.0 while activity (stoichiometry) for amino-group acceptors was determined in the presence of 5 mM taurine, 0.1 mM pyridoxal 5′-phosphate in 100 mM Tris/HCl, pH 9.0. Disappearance of taurine and formation of products were measured by HPLC as detailed in Materials and Methods.

Amino-group donor	Activity (%)	
Taurine	100	
Hypotaurine	218	
β-Alanine	37	
Cysteine	4.0	
3-Aminopropanesulfonate	3.6	
Cysteate	0.2	
Glutamate	0.2	
2-Aminoethanephosphonate	0.1	
Amino-group acceptor	**Disappearance of taurine (mM)**	**Product (mM)**
None	0	None
Pyruvate	1.4	Alanine (1.3)
2-Ketobutyrate	0.5	2-Aminobutyrate (0.1)
Oxaloacetate	1.0	Not identified (≈ 0.8)
2-Oxoglutarate	0	None
Phenylpyruvate	0	None

In confirmation of data given above, the values in Table 2 A generally indicate unit stoichiometry for the transamination.

The regeneration of pyruvate as amino-group acceptor for the Tpa transamination reaction is performed by alanine dehydrogenase, which catalyses the oxidative deamination of alanine yielding pyruvate and ammonia (Fig. 1). The apparent K_m value for pyruvate of the Tpa (0.82 mM) is lower than that of the alanine dehydrogenase for alanine (1.6 mM) [22], which allows sufficient regeneration of the amino-group acceptor for transamination of taurine.

Tpa from *B. wadsworthia* has significantly higher activities with (hypotaurine and) taurine than with β-alanine, appropriate to its role in taurine dissimilation. In contrast, 'taurine:2-oxoglutarate aminotransferase' of *A. superficialis* transaminates hypotaurine, DL-aminoisobutyrate and β-alanine with significantly higher activities than taurine, and that enzyme is induced by β-alanine [29]. Therefore, as in the case of ω-amino-acid aminotransferase [8], 'taurine:2-oxoglutarate aminotransferase' seems really to be a miss-named ω-amino acid:2-oxoglutarate aminotransferase.

Sequence analysis

1.1 kbp of the gene encoding Tpa was amplified with oligonucleotide primers deduced from the N-terminal and internal amino-acid sequences and sequenced. By adaptor-ligated PCR we amplified and sequenced the gene and surrounding regions. A total DNA fragment of 3.2 kbp was sequenced. The open reading frame (1371 bp) encodes a protein consisting of 456 amino acids with a molecular mass of 49.7 kDa, which is in good agreement to the value of 51 kDa determined for the purified Tpa by SDS/PAGE (Fig. 3). The theoretical isoelectric point was estimated to be 5.9. The three amino-acid sequences determined for the purified protein were observed in the derived sequence. The G + C content (59.3 mol %) was higher than the overall G + C content of *B. wadsworthia* (39–40 mol %) [13]. Putative σ^{70} promotor regions were located 24 bp upstream of the translational start of the Tpa and a stem-loop-stem motif was found 12 bp downstream of the stop codon.

The deduced amino-acid sequence of the Tpa was 33%, 32%, and 31% identical to those of the diaminopelargonate-aminotransferase from *Bacillus subtilis* [33], ω-amino acid:pyruvate aminotransferase from *Pseudomonas putida* [31], and acetylornithine aminotransferase from *Escherichia coli* [34]. The latter enzymes belong to subgroup II of the aminotransferases, whose common substrate feature is a distal amino group which undergoes the transamination [35]. Sequence similarities and the reaction catalysed thus indicate that Tpa also belongs to subgroup II of the aminotransferases.

In contrast to the relatively low identity of ≤ 33% of the deduced amino-acid sequence of the *tpa* gene to other aminotransferases, the other characterized enzymes involved in taurine degradation of *B. wadsworthia*, alanine dehydrogenase (Ald) [22] and the α and β subunits (DsrA and DsrB) of dissimilatory sulfite reductase [36] were 57%, 84% and 83% identical, respectively, to their corresponding enzymes. All of these genes (*tpa*, *ald*, *dsrAB*) have a higher G + C content (59.3–61.9 mol%) than the overall G + C content of *B. wadsworthia* (39–40 mol%) [13]. Analysis of the codon usage of these genes (*tpa*, *ald* [22], *dsrAB* [36] reveals that *B. wadsworthia* had a bias toward CGC/T as arginine codon, GCC as alanine codon, GGC as glycine codon, TCC as serine codon, and GTC/G as valine codon.

127 bp downstream of the *tpa* gene, a putative, truncated ORF (685 bp) was found whose deduced amino-acid sequence showed similarities to alcohol dehydrogenases. It was 42, 38, 36 and 34% identical to AttL from *Agrobacterium tumefaciens* [37], alcohol dehydrogenase GbsB from *Bacillus subtilis* [38], NAD-dependent methanol dehydrogenase from *Bacillus methanolicus* [39], and alcohol dehydrogenase (ADH4) from *S. cerevisiae* [40].

We located a truncated ORF 121 bp upstream of the *tpa* coding region. It exhibited high similarities to alanine dehydrogenases, and the complete *ald* gene has now been sequenced [22]. A putative terminator sequence was found downstream of the stop codon of the *ald* gene, just as another

was found downstream of the *tpa* gene (see above). We believe that these terminators indicate separate regulation of these two genes, because growth with pyruvate gives the highest expression of the *tpa* gene (see above), whereas pyruvate represses and taurine induces expression of the *ald* gene [22]. In contrast to Tpa of *B. wadsworthia*, the assimilatory Tpa of *C. pasteurianum* C1 is induced by taurine and repressed by sulfate [9].

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