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The taurine biosynthetic pathway of microalgae

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ABSTRACT

Taurine (2-aminoethanesulfonic acid) is an amino acid-like compound widely distributed in animals and an essential nutrient in some species. Targeted metabolomics of marine and fresh water microalgae combined with medium supplementation identified biosynthetic pathway intermediates and necessary catalytic activities. Genomic analysis was then used to predict the first taurine biosynthetic pathway in these organisms. MRM-based electrospray ionization (ESI) LC–MS/MS analysis demonstrated that taurine is synthesized using a carbon backbone from L-serine combined with sulfur derived from sulfate. Metabolite analysis showed a non-uniform pattern in levels of pathway intermediates that were both species and supplement-dependent. While increased culture salinity raised taurine levels modestly in marine alga, taurine levels were strongly induced in a fresh water species implicating taurine as an organic osmolyte. Conservation of the synthetic pathway in algae and metazoans together with a pattern of intermittent distribution in other lineages suggests that it arose early in eukaryotic evolution. Elevated levels of cell-associated taurine in algae could offer a new and biorenewable source of this unusual bioactive compound.

1. Introduction

Taurine, 2 aminoethanesulfonic acid, is a unique sulfur-containing free amino acid because it does not form peptide bonds with other amino acids. Consequently, taurine can be an abundant small molecule in animal blood and tissues [1–3]. There are multiple biosynthetic pathways for taurine synthesis in animals (Fig. 1). Some species utilize cysteine and/or methionine to synthesize taurine referred to here as the CDO/CSAD pathway [1–3]. In this pathway, cysteine is first oxidized to cysteine sulfinic acid by cysteine dioxygenase (CDO) [4] that is then decarboxylated to form hypotaurine by cysteine sulfinic acid decarboxylase (CSAD) or glutamate decarboxylase (GAD) [5]. Hypotaurine undergoes oxidation to form taurine in what is thought to be a spontaneous process as no enzymes have been reported that catalyze this reaction [1,3]. A second animal pathway referred to here as the Serine/Sulfate pathway, occurs in chick livers and developing chick embryos and is instead repressed by cysteine [6]. This pathway utilizes inorganic sulfate to form sulfonate-2-aminocrylate followed by cysteate in a reaction coupled to the conversion of adenosine-3′-phosphate-5′-phosphosulfate (PAPS) to adenosine-3′,5′-diphosphate (PAP). Cysteate is then decarboxylated to form taurine [7]. Bacterial taurine synthesis remains unclear though exogenous cysteine sulfinic acid supplementation enables accumulation of intracellular taurine indicating the presence of a CSAD homolog [8]. In many bacteria, taurine catabolism involves deamination of taurine to sulfoacetaldehyde followed by desulfonation producing acetyl-phosphate and sulfite [9]. Finally, acetyl-phosphate is either assimilated via the Krebs cycle or converted to acetate via substrate level phosphorylation [9]. Plants and fungi are currently not known to synthesize taurine, however a growth stimulatory response has been reported for plants [10].

In contrast to animals, plants, and bacteria, algal taurine metabolism is understudied. The presence of taurine has been confirmed in selected dinoflagellates, prasinophytes, rhodophytes and diatoms, but not in prymnesiophytes or chlorophytes [11–14]. However in most cases taurine identification was based purely on migration with standards during HPLC and not more on definitive and sensitive methods such as mass spectrometry. Analysis of heterotrophic dinoflagellates suggests that taurine can be correlated with chlorophyll *a* [15,16] in some as yet unknown manner. However, the metabolic pathway(s) resulting in taurine formation in algae has not been reported. In the present study, algal species were selected based on the following criteria: i) prior reports regarding production of taurine by the species; ii) the availability of a genome sequence; and iii) the potential applications of the algal species. Here a combination of metabolomics combined with chemical supplementation and genomic analyses enabled the identification of

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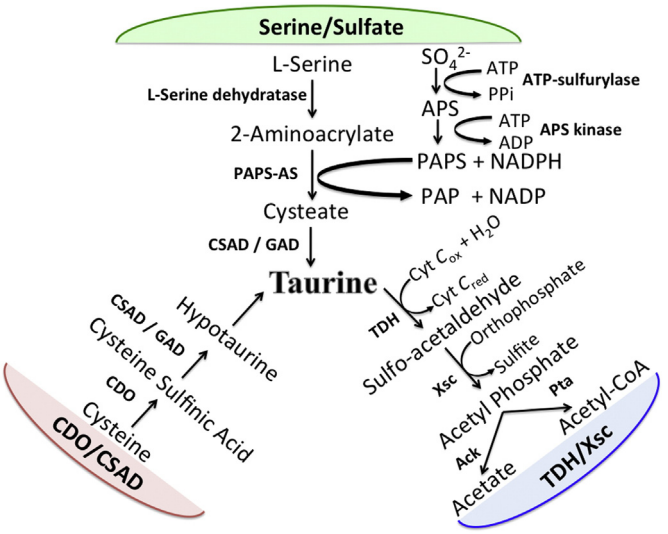

Fig. 1. Taurine metabolism in various biological systems (CDO — cysteine dioxygenase, CSAD — cysteine sulfinic acid decarboxylase, GAD — glutamate decarboxylase, TDH — taurine dehydrogenase, Xsc — sulfoacetaldehyde acetyltransferase, Ack — acetate kinase, Pta — phosphotransacetylase, ATP-sulfurylase — ATP:sulfate adenylyltransferase, APS kinase — adenylyl-sulfate kinase, PAPS-AS — 3′-phosphoadenylyl sulfate:2-aminoacrylate C-sulfotransferase). The CDO/CSAD pathway is found in animals [1–5]; the Serine/Sulfate pathway is found in developing chick embryos [6], chick liver [7] and microalgae (present study); the XDH/Xsc pathway is found in bacteria [8,9].

the first taurine biosynthetic pathway in algae. These results may encourage the use of algae as a biological source of taurine.

2. Materials and methods

2.1. Microalgae strains and culturing

Chlamydomonas reinhardtii CC124 (laboratory collection), *Tetraselmis* sp. (PLY 429, Reed Mariculture Inc., USA), and *Ostreococcus tauri* (RCC 745, Roscoff Culture Collection, France) were used for experimental studies, and were grown in TAP [17], f/2 modified [18], or K medium [19], respectively. All cultures were grown in 25 mL of their respective media in 125 mL shake flasks. The conditions were maintained at 25 °C under continuous illumination (light intensity ~50 μmol photons m^{-2} s^{-1}) and agitation (180 rpm). The growth curves were prepared by plotting respective OD_{550} values with time. All the experiments were performed under aseptic conditions in triplicate. Samples were taken for dry weight analysis and centrifuged at 12,000 ×*g* for 15 min. Pellets were dried in a SpeedVac (Savant, USA) and then used for dry weight analysis.

2.2. Sulfur compound or serine supplementation

Ten different sulfur compounds were investigated for their effect on growth physiology and taurine levels in *O. tauri*. These compounds were chemical grade quality purchased from Sigma-Aldrich, USA. The stock solutions of filter sterilized sulfur compounds were aseptically added to sterile K-medium at 6 mM final concentration.

2.3. Salinity effect

Sea salt (Sigma-Aldrich, USA) consisted of: chloride (19.29 g/L), sodium (10.78 g/L), sulfate (2.66 g/L), potassium (420 mg/L), calcium (400 mg/L), bicarbonate (200 mg/L), strontium (8.8 mg/L), boron (5.6 mg/L), and bromide (56 mg/L). TAP (fresh water algal medium) contained 0% sea salts, whereas f/2 modified media (marine microalgal medium) had 1.6% (w/v) sea salts (both of these media were used as controls). The sea salts were added in 1.6% (w/v) increments to the salts present in the respective media. All solutions were autoclaved prior to use. Cell pellets were stored at −20 °C until taurine analysis was performed.

2.4. Extraction of intracellular amino acids

Total intracellular amino acid pools were extracted from selected microalgae grown in their respective media. The cells were centrifuged at 12,000 ×*g* and then suspended in 1 mL HPLC grade water (VWR). Samples were sonicated five successive times for 30 s followed each time by a 30 s interval on ice. Sonicated samples were then incubated at 65 °C for 1 h and then centrifuged at 15,600 ×*g* at 4 °C for 20 min. The supernatant was then used for LC–MS/MS analysis. All algal samples were processed in triplicate.

2.5. Amino acid analysis using LC–MS/MS

Sulfur containing amino acids and related compounds was quantified from algal extracts with a Shimadzu UFLC-XR (Shiamadzu Scientific Instruments, Inc., Columbia, MD) and an AB SCIEX Q-Trap 4000 LC–MS/MS (AB SCIEX, Framingham, MA) electron spray ionization detector. Isocratic chromatographic separations were achieved within 8 min using a Zorbax Eclipse plus 3.5 μm, 150 × 4.6 mm reverse phase C18 column (Agilent Technologies, Santa Clara, CA) and a 10 mM aqueous ammonium acetate solution (pH 9.3) [20] at a constant flow rate of 0.3 mL min^{-1} and 40 °C column temperature. The column was washed for 2 min with methanol between sample runs and allowed to re-equilibrate for 3 min prior to the next injection to remove photosynthetic pigments. Taurine, hypotaurine, cysteic acid, methionine, cysteine, and norvaline were analyzed in positive ionization mode. Cysteine sulfinic acid was analyzed in negative mode (SI_01 Figs. S1–S9). Multiple reaction monitoring (MRM) was used to obtain peak areas. Optimal depolarization potentials, collision energies, precursor and product ions were obtained experimentally through direct MS infusion experiments (Table 1), and the respective values for standard cysteine, taurine, norvaline, and methionine corresponded with published values [21]. MRM peak areas were compared with calibration graphs of known standard concentrations measured before and after each run. Quantities were extrapolated using norvaline to calculate the internal response factor of amino acids during sample processing.

2.6. Bioinformatic analysis

A phylogenetic tree was constructed using 18s rRNA sequences of microalgae obtained from various taxonomic divisions available in the NCBI database. Sequence alignments and phylogenetic analysis were performed using the neighbor-joining method implemented in MEGA 6.0 software [22] with 1000 bootstrap replicates. The Domain Enhanced Lookup Time Accelerated BLAST (DELTA-BLAST, NCBI) [23] was used to identify enzymes involved in all possible taurine biosynthetic pathways

Table 1
Results of optimal multiple reaction monitoring (MRM), depolarization potentials (DP), collision energies (CE), precursor and product ions (transition) empirically derived from MS infusion experiments (CYS: cysteine; CSA: cysteine sulfinic acid; CA: cysteic acid; HYP: hypotaurine; TAU: taurine; NVA: norvaline).

	MRM transition	DP	CE
HPT	110 > 92	10	18
NVA	118 > 72	10	18
CYS	122 > 76	15	20
TAU	126 > 108	15	8
MET	150 > 104	10	14
CA	170 > 124	50	5
CSA	172 > 155	−100	−20