Uptake and Utilization of Sulfonic Acids in the Cyanobacterial Strains *Anabaena variabilis* and *Plectonema* 73110

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Growth of several cyanobacteria was examined on ethane sulfonate and taurine as only sulfur source. Comparing two strains with differential utilization of sulfonic acids (*Anabaena variabilis* and *Synechococcus* 6301) demonstrated that actual growth was coupled to the presence of an active sulfonate transport system due to species specific properties and nutritional conditions. Sulfonate uptake in *Anabaena variabilis* was characterized by a pH optimum of 6.5, a structural specificity for sulfonates, missing Na^+ dependence, and phosphate stimulation. Radiolabeled ethane sulfonate and taurine was metabolized to products of normal sulfur metabolism. Also considerable amounts of ^{35}S-labeled volatiles (mercaptanes and sulfide) could be detected, suggesting a degradation mechanism *via* reduction to mercaptanes and cleavage of the C–S bond.

Introduction

Sulfonic acids are reported as naturally occurring and man-made chemicals in our environment [1]. Especially the β-amino acid taurine (2-aminoethane sulfonate) has a wide distribution in animal or plant systems [2, 3]. The presence of taurine was documented also in bacteria [4–6] showing taurine as a nearly ubiquitous compound. In addition, sulfoacetate, sulfolactate, isethionate, cysteic acid, sulfoacetaldehyde, sulfopropanediol, 2-mercaptoethane sulfonate (coenzyme M) and sulfonolipids are produced from living organisms in relatively large quantities and have to be degraded in nature to balance the global sulfur cycle [7–14].

So far, uptake and degradation of sulfonic acids were examined mainly in bacteria [15–18], fungi [19, 20], and animal systems [21–24]. Recently sulfonic acid catabolism has been described also in photosynthetic organisms as various green algae could utilize ethane sulfonic acid as only sulfur source for growth [25]. Further studies confirmed that ethane sulfonate was taken up only in those strains utilizing sulfonates for growth. Transport and metabolization of ethane sulfonate and taurine was characterized in the green alga *Chlorella fusca* suggesting identical mechanisms for uptake and metabolism of these sulfonic acids [25, 26].

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As the cyanobacterium *Synechococcus* 6301 did not grow on sulfonic acids as only sulfur source [27], it was of interest to know if this property was a specific feature of the *Synechococcus* strain used or if it could be generalized to cyanobacteria. To our knowledge no further information was available on function, uptake and metabolism of sulfonic acids in cyanobacteria. The following study was initiated to analyse those problems in selected strains, suggesting a possible role for cyanobacteria in the biodegradation of sulfonic acids.

Materials and Methods

Organisms

The following cyanobacterial strains were obtained from the algal collection of Institute Pasteur (Paris): *Anabaena cylindrica* 7122, *Anabaena variabilis* 7129, *Colothrix* 7101, *Nostoc* 6310, *Plectonema* 73110, *Pseudanabaena* 7408, *Spirulina* 7318, *Synechococcus* 6301 and 6312, and *Xenococcus* 7307; *Anabaena variabilis* (strain P. Wolk) was a gift of Dr. Lockau (Regensburg). Cultures were grown in the BG-11 medium [28] in 750 ml Pirson flasks at 27 °C and 10,000 lux with normal air for aeration. Sulfur sources for growth were used at 0.3 mM concentrations.

Uptake assays

The transport capacity for [^{35}S]ethane sulfonate and taurine was measured as described earlier [26].

Chemical fractionation studies

Algal cells were incubated for 1 h in ethane [^{35}S]sulfonate or [^{35}S]labeled taurine. The incorporated radioactivity was analyzed as previously described [26]. For detection of volatile [^{35}S]mercaptanes the Conway technique was used. After addition of 100 µmol ethylmercaptane as carrier, [^{35}S]labeled mercaptanes were expelled by 0.01 N HCl from intact cells, trapped in 1 ml CuCl (1 g/10 ml H_2O) and counted in a liquid scintillation counter. Possible formation of [^{35}S]sulfide was analyzed using 100 µmol of non-labeled Na_2S as carrier and trapping volatiles after addition of 1 N HCl in 1 N zink acetate using the Conway technique.

Chemicals

[^{35}S]taurine was obtained from Amersham-Buchler (Braunschweig, W. Germany) and purified by thin-layer chromatography on silica gel GF_{253} (Merck, Darmstadt, W. Germany) using the solvent system ethanol:H_2O = 70:30. [^{35}S]labeled ethane sulfonic acid was synthesized and purified as described earlier [26]. All chemicals not mentioned were obtained from Merck (Darmstadt, W. Germany).

Results

Utilization of ethane sulfonate and taurine by cyanobacteria

Several cyanobacteria were tested for their ability to grow on ethane sulfonate and taurine as only sulfur source. Growth on taurine and ethane sulfonic acid could be detected in *Anabaena variabilis, Plectonema* 73110, and *Anabaena cylindrica* 7122 [24]; no growth on these two sulfonic acids was found for *Synechococcus* 6301 and 6312, *Xenococcus* 7037, *Anabaena variabilis* 7120, *Nostoc* 6310, *Pseudanabaena* 7408, *Spirulina* 6313, and *Calothrix* 7101. Thus, the ability for sulfonate uptake and degradation seems to be present in cyanobacteria. For further comparative studies one strain with good growth on sulfonic acids was selected (*Anabaena variabilis* strain P. Wolk) and set against *Synechococcus* 6301 which could not use sulfonates at all.

Occurrence of sulfonate uptake in Anabaena variabilis and Synechococcus 6301

Both *Anabaena variabilis* (good growth) and *Synechococcus* 6301 (no growth) were tested for ethane sulfonate and taurine uptake under different sulfur nutrition. Table I demonstrates sulfonate uptake in *Anabaena variabilis* according to specific cultivation conditions: growth on ethane sulfonate, taurine, and sulfur limitation. However sulfonate uptake was not constitutive, as sulfate-grown cultures failed to transport ethane sulfonate or taurine. Sulfonate transport was also detected in sulfur starved cultures, suggesting a different mechanism than substrate induction.

Table I. Uptake of sulfate, ethane sulfonate and taurine in *Anabaena variabilis*. Algae were grown for 5 days using sulfur sources as indicated in 0.3 mM concentration. Uptake rates for [^{35}S]labeled ethane sulfonate, [^{35}S]taurine and [^{35}S]sulfate were assayed in a Warburg apparatus using the following conditions: 27 °C, 4500 lux, 30 min and a final concentration of 0.3 mM for each sulfur compound in the assay. A specific activity of 65000 Bq/nmol was used in all cases. Data are expressed as 10^{-9} nmol × cell^{-1} × h^{-1}.

Sulfur nutrition	Sulfate uptake	Ethane sulfonate uptake	Taurine uptake
Sulfate	62	0	0
Ethane sulfonate	686	86	191
Taurine	646	78	138
S-deficiency	466	184	144

In contrast, no sulfonate transport was detected in *Synechococcus* 6301, a strain unable to utilize sulfonic acids for growth. Apparently no uptake of ethane sulfonate or taurine could be detected under all nutritional conditions tested although an increased sulfate uptake capacity during sulfur starvation was measured. Thus sulfonate utilization seems to be coupled to the individual ability for a sulfonate uptake (carrier) system.

The data presented in Table I and II demonstrate further that taurine and ethane sulfonate uptake was always correlated with increased sulfate uptake capacity, a signal for sulfur starvation in green algae.

Table II. Uptake measurements for sulfate, ethane sulfonate and taurine in *Synechococcus* 6301. Identical conditions as in Table I were used.

Sulfur nutrition	Sulfate uptake	Ethane sulfonate uptake	Taurine uptake
Sulfate	4.5	0	0
Ethane sulfonate	6.6	0	0
Taurine	5.9	0	0
S-deficiency	8.0	0	0

Effect of pH on taurine uptake in Anabaena variabilis

Taurine uptake was markedly affected by the pH of the uptake medium in the range of 5.0 to 11.5. A pH of 6.5 was found to be optimal using 0.01 M potassium phosphate buffer (Fig. 1). This pH-optimum for taurine uptake is distinct from that of the sulfate permease (9.5) showing a clear difference between sulfate and sulfonate uptake.

Substrate specificity of taurine uptake in Anabaena variabilis

The specificity was tested by competition experiments using various sulfonates, amino acids and sulfate in a 10-fold higher concentration to taurine (Fig. 2). Obviously all sulfonic acids tested competed with taurine uptake despite of different functional groups in the molecules. However all amino acids analyzed failed to diminish taurine transport markedly; also the sulfate ester aminoethyl sulfate and sulfate did not affect taurine influx; thus the specificity of taurine uptake seems to be limited to the sulfonate group itself. *Anabaena variabilis* can further distinguish between sulfonate and carboxylic groups, as β-alanine (the carboxylic analogue of taurine) failed to compete for taurine uptake.

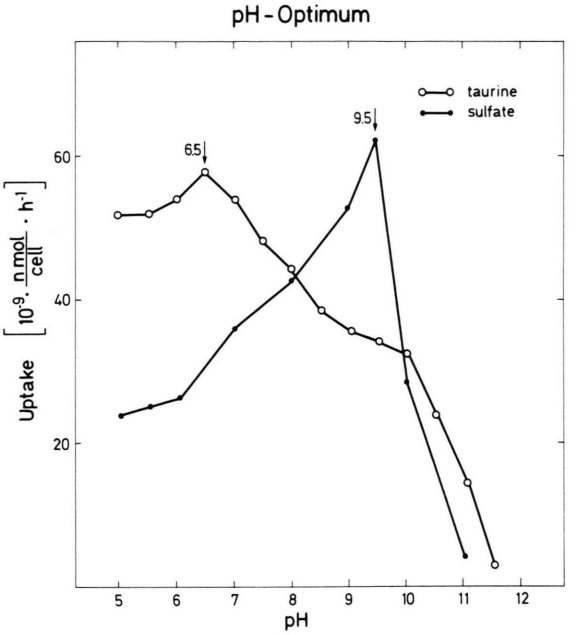

Fig. 1. pH-Dependence of taurine uptake in *Anabaena variabilis*. Washed cells of taurine-fed *Anabaena variabilis* were incubated in 2 ml of 0.01 M potassium phosphate buffer pH 5.0–11.5 for 30 min. Taurine uptake was measured according to Table I.

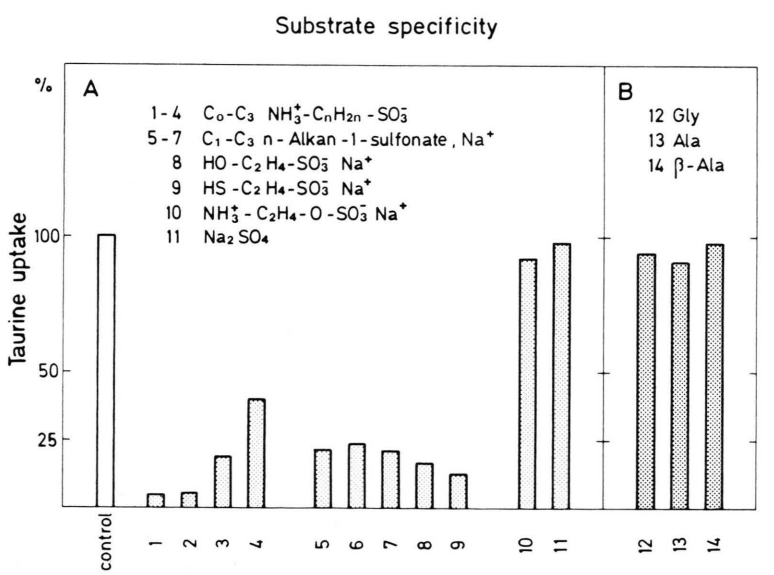

Fig. 2. Effect of structural analogues on taurine uptake in *Anabaena variabilis*. Washed taurine-grown algae were preincubated for 5 min in the presence of the unlabeled and 3 mM concentrated analogue before adding [^{35}S]taurine to the incubation medium. Principal conditions as in Table II. All compounds were used as sodium salts with the exception of the amino acids.

Effect of sodium ions on taurine uptake in Anabaena variabilis

Taurine transport was independent from the actual Na^+-concentration in the range of 0.1–10 mM during incubation (Table III), since uptake was not stimulated by enhanced Na^+-concentration in the medium. However the phosphate buffer used increased taurine transport markedly, an effect which was already observed for sulfonate uptake in *Chlorella fusca* [26].

Table III. Effect of the sodium concentration on taurine uptake in *Anabaena variabilis*. Taurine-grown algae were washed with sulfur-free growth medium and preincubated for 5 min in the presence of 0.1–10 mM NaCl using 1 mM KH_2PO_4/K_2HPO_4-buffer pH 7.8. Principal conditions as in Table I.

Na^+-concentration	Taurine uptake [%]
BG-11 medium (18.5 mM Na^+)	100
KH_2PO_4/K_2HPO_4 buffer	165
buffer + 0.1 mM NaCl	172
buffer + 1 mM NaCl	170
buffer + 10 mM NaCl	175

Metabolism of sulfonic acids in Anabaena variabilis

After incubation for 1 h in 0.3 mM ethane [^{35}S]sulfonate or taurine the incorporated radioactivity was further examined as described in Materials and Methods. More than 90% of the radioactivity taken up was recovered in the water-soluble fraction (ethane sulfonate/taurine, sulfate, cysteine, methionine, glutathione), 2% was found in the methanol/chloroform fraction (sulfolipid) and 5% remained unsoluble. Considerable amounts of [^{35}S]-labeled mercaptanes could be detected after incubation in [^{35}S]ethane sulfonate. Also [^{35}S]labeled H_2S was found using intact cells suggesting a mechanism of sulfonate metabolism *via* reduction to the level of mercaptanes and further cleavage of the C–S bond to sulfide.

Discussion

Our studies demonstrated the growth of several cyanobacteria on sulfonic acids utilizing the nutritional potential of these unusual sulfur sources. However, not all cyanobacteria tested possessed sulfonate utilization and a clear heterogeneity within taxonomic classifications was shown.

To clarify whether these differences are caused by missing uptake capacities and/or missing degradation capacities ethane sulfonate and taurine transport was measured in two cyanobacteria different in sulfonate growth. A comparison of the uptake capacities of *Anabaena variabilis* (+) and *Synechococcus* 6301 (–) confirmed that sulfonate utilization is coupled to the presence of an active sulfonate transport system. No sulfonate uptake was detected in *Synechococcus* 6301 under all conditions tested, whereas uptake was present in *Anabaena variabilis* grown under taurine, ethane sulfonate or S-limited conditions. However, ethane sulfonate and taurine uptake were not constitutive as sulfate grown algae did not transport sulfonic acids excluding unspecific membrane diffusion. In addition, ethane sulfonate/taurine uptake was not caused *via* substrate induction (the presence of sulfonic acids in the medium) as sulfonate uptake was also found in sulfur-starved cultures. Thus sulfonate transport seems to develop specifically in response to sulfate limitation according to previous studies using *Chlorella fusca* [26]. Enhanced sulfate uptake as a signal of sulfate shortage was measured in all cultures without sulfate addition independently of sulfonate uptake. Obviously enhanced sulfate (and sulfonate uptake) can be interpreted as a specific response to sulfate limitation in cyanobacteria as well [29–31].

Further studies demonstrated the existence of a distinct carriermediated transport system ("permease") in sulfonate grown *Anabaena variabilis*. Taurine uptake was pH dependent and highly specific for sulfonic acids. Recognition of possible substrates seems to be limited to the sulfonate group itself, whereas the presence of other functional groups in the sulfonic acid molecule (HO–, HS–, NH_2–, HOOC–) or chain length did not influence the affinity for this transport system. Obviously taurine transport in *Anabaena variabilis* is mediated by a "sulfonic acid permease" demonstrating specific binding sites for the sulfonate group with no further charge or size limitation, thus being analogous to the sulfonate permease of *Chlorella fusca* [26].

The uptake mechanism of taurine in *Anabaena variabilis* is unknown, although Na^+-dependent transport processes can be excluded. Neither the absence of sodium in the assay nor the addition of Na^+ affected taurine uptake significantly. Also the presence of monensin, known to block Na^+-linked

transport systems, affected taurine uptake relative moderately compared to other procaryotic systems [15].

Thus, taurine uptake in *Anabaena variabilis* under sulfate/sulfur limitation resembles accurately sulfonate uptake in *Chlorella fusca* referring to structural specificity, phosphate stimulation and missing Na^+-dependence [25, 26]. Great differences are shown to taurine uptake in bacteria [15] or animal systems so far analyzed [21, 23, 24]: In both heterotrophic groups taurine uptake was constitutive, Na^+-dependent and highly specific for β-amino acids (animals) or taurine itself (bacteria). The enclosure of other sulfonic acids without amino groups in taurine transport processes was never described and seems to be limited to permeases in cyanobacteria and chlorophyta. Obviously sulfonate uptake under sulfur limitation is an evolutionary old mechanism and was retained in various photosynthetic organisms as a favourable attribute.

Most of the radioactivity taken up appears in products of sulfur metabolism suggesting that sulfonic acids readily enter normal metabolic pathways in *Anabaena variabilis*. After incubation with [^{35}S]ethane sulfonate/taurine the following radiolabeled compounds were detected: cysteine, methionine, glutathione, sulfate and sulfolipids. Also considerable amounts of [^{35}S]labeled volatiles (mercaptanes and sulfide) could be detected in intact cells suggesting degradation of sulfonic acids *via* reduction to mercaptanes and C–S bond cleavage at this niveau. Thus sulfonic acid catabolism in *Anabaena* would be distinct from all degradation mechanisms studied so far: In bacteria the sulfonic acid taurine is catabolized by transamination and desulfuration reactions leading to sulfite and acetate [16–18, 32]. In green algae sulfonic acids are degraded to sulfate and the corresponding alcohol [26, 33]. In animal systems evidence for taurine degradation is tenuous showing sulfonates as inert compounds with no further metabolism within the tissue.

Besides heterotrophic bacteria and fungi also algae and cyanobacteria may play a role in the biodegradation of sulfonic acids. The importance of sulfonate degradation by cyanobacteria should be discussed in two aspects: 1) The recycling of sulfonate-bound sulfur also by autotrophic organisms to balance the global sulfur cycle. 2) A favourable mechanism to overcome sulfur starvation conditions in the aquatic environment. Thus the apparent "unspecificity" for use of sulfonic acids may be beneficial to cyanobacteria and green algae for utilization of sulfonates produced and released in nature.

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