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Characterization of the non-constitutive ethanesulfonate uptake in *Chlorella fusca*

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Ethanesulfonate entered *Chlorella fusca* via a temperature-, pH-, energy-, and concentration-dependent system ('permease'). Transport was unidirectional and followed hyperbolic saturation kinetics with a Michaelis-Menten constant of $5 \cdot 10^{-5}$ M. Uptake was substantially inhibited by structural analogues, indicating a high specificity for the sulfonate group in the compounds tested. Furthermore the permease could clearly distinguish between other monovalent anions, monocarboxylates or sulfate, indicating a distinct uptake system in this alga. The negative effect of several metabolic inhibitors and SH-group reagents on ethanesulfonate uptake was evident. Ethanesulfonate uptake could be enhanced by polyvalent cations apparently via reduction of the surface potential and could be strongly stimulated by addition of phosphate ions to the medium. Radioactive labelled ethanesulfonate was rapidly metabolized to sulfate, the main and probably the first degradation product of ethanesulfonic acid in *Chlorella fusca*.

Introduction

Sulfonic acids are naturally occurring and man-made chemicals in our environment [2]. Especially 6-sulfo-D-quinovose as part of the sulfolipids and the β-amino acid taurine (2-aminoethanesulfonate) are produced by living organisms in relatively large quantities and have to be degraded in order to balance the global sulfur cycle. Also man-made sulfonic acids (detergents, various drugs, dyestuffs, pesticides or by-products of industrial processes) are frequently found in industrial or common waste waters and have to be mineralized in nature as well.

So far, degradation of sulfonic acids and their subsequent utilization as sources for carbon, nitrogen or sulfur are commonly known only for heterotrophic organisms [7,13,14]. Recently sulfonic acid catabolism has been described also in photosynthetic organisms, since various green algae and cyanobacteria could utilize ethanesulfonic acid as only sulfur source for growth [3]. Further studies confirmed that ethanesulfonic acid was taken up only in those strains utilizing ethanesulfonic.

This led to a study of the uptake and metabolism of ethanesulfonic acid in the green alga *Chlorella fusca*, presenting a specific uptake system for sulfonic acids. As shown previously, this alga could utilize sulfonic acids as sulfur source growing

Abbreviations: CCCP, carbonylcyanide *m*-chlorophenylhydrazone; DCCD; *N*,*N*′-dicyclohexylcarbodiimide; DCMU; (3-(3,4-dichlorophenyl))-1,1-dimethylurea; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (dibromothymoquinone); PCBMS, *p*-chloromercuribenzylsulfonate; PMSF, Phenylmethylsulfonyl fluoride.

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comparably to sulfate in case of ethanesulfonic acid [2]. However, sulfate-grown algae did not import ethanesulfonate, excluding unspecific membrane transport phenomenons. Ethanesulfonate uptake was depressed under sulfate/sulfur starvation conditions within 2–3 h and was repressed soon after addition of sulfate.

The following study will characterize the ethanesulfonate uptake system of the green alga *Chlorella fusca* to be an active, carrier-mediated permease.

Materials and Methods

Organism

Chlorella fusca 211-8b from the algal collection of Göttingen was cultured as described earlier at 28°C, 10.000 lux, and gassed with normal air [12]. The algal cells used for uptake studies were harvested in the exponential phase of growth usually 4–5 days after inoculation.

Uptake assays

Cultures grown for 4–5 days were harvested in 10 ml samples, washed in the corresponding sulfur-free medium and incubated in 2 ml of medium supplemented with ethanesulfonic acid (sodium salt). The concentration of ethanesulfonate was adjusted to a final concentration of 0.3 mM and a specific radioactivity of 65000 Bq/nmol. Algae were incubated for 5–90 min in a Warburg vessel at 27°C and 4.500 lux and analyzed afterwards for radioactivity. The samples were washed in sulfur-free medium and counted finally in a liquid scintillation counter using Rotiszint 22. For metabolic studies a specific activity of radiolabelled ethanesulfonate of 650000 Bq/nmol was used.

Chemical fractionation studies

Algal cells were incubated for one hour in ethane[^{35}S]sulfonate and the incorporated radioactivity was analyzed afterwards as follows:

(a) Preparation of ^{35}S-algal extracts. Incubated cells were centrifuged and washed with sulfur-free medium several times. After adding 1 μmol non-labelled carrier of sodium ethanesulfonate, sodium sulfate, cysteine, methionine, and glutathione, the cells were killed by addition of boiling 80% ethanol/0.01 M acetic acid and extracted for 5 min at 80°C in the water bath. The suspension was centrifuged to yield a supernatant fluid containing more than 80% of the radioactivity taken up. This procedure was repeated twice. Afterwards the pellet was resuspended in chloroform/methanol (2 : 1, v/v) at room temperature for 2 × 5 min each and washed twice with deionized water. The supernatant solutions obtained from each extraction step were collected, the remaining pellet was dissolved and counted for radioactivity. The combined soluble extracts were fractionated into the chloroform-soluble fraction by addition of chloroform. After shaking and separating the compounds into two phases the methanol-water phase was removed, the chloroform phase and the interfacial emulsion were washed two times more with 1.5 volumes of water. The water washes were combined with the methanol-water probe to yield the water-soluble substances. Both fractions were reduced in volume to approximately 0.5 ml, counted and subjected to further analysis.

(b) Analysis of water-soluble compounds. The water-soluble radioactivity was submitted to chromatography and electrophoretic analysis on silica gel GF 253 (Merck, Darmstadt, F.R.G.). The fraction was divided into halves. (a) One part was chromatographed at room temperature in the solvent system isopropanol/NH_3/H_2O (20 : 1 : 4, v/v) determining the R_F-values of ethane [^{35}S]sulfonate (0.57) and [^{35}S]sulfate (0.0) by co-chromatography and counting radioactivity. (b) The second aliquot was electrophoreted in 0.1 M citrate buffer (pH 5.8) at 500 V and 14°C for 30–40 min.

After drying at 110°C the thin-layer plates were either counted for radioactivity or developed with ninhydrin reagent at 110°C to detect the unlabelled comigrated amino acids. For counting, the silica gel was separated in 1 cm strips and eluted with water. Aliquots of the dissolved material were counted in the liquid scintillation counter after adding the cocktail Rotiszint 22.

(c) Analysis of chloroform-soluble compounds. Sulfolipids as the only radioactive chloroform-soluble compound were assayed by thin-layer chromatography with the solvent system acetone/benzene/H_2O (45 : 15 : 4, v/v) and using lipid standards as references.

Chemicals

^{35}S-labelled carrier-free sulfate and SO_2 were obtained from Amersham-Buchler (Braunschweig, F.R.G.). ^{35}S-labelled ethanesulfonic acid was synthesized using a modified technique of Houlton and Tartar [6]. Sulfate, sulfite, and other impurities were removed by thin-layer chromatography on silica gel GF253 (Merck, Darmstadt, F.R.G.) using the solvent system iso-propanol/NH_3/H_2O (20 : 1 : 4, v/v). PCMBS, PMSF, and DBMIB were purchased from Sigma (St. Louis, MO, U.S.A.), DCCD from Fluka AG (Buchs, Switzerland), CCCP from Serva (Heidelberg, F.R.G.) and Rotiszint 22 from Roth (Karlsruhe, F.R.G.). All chemicals not mentioned were obtained from Merck (Darmstadt, F.R.G.).

Results

Linearity of ethanesulfonate uptake

(a) Time-course. Ethanesulfonate uptake at 0.3 mM by *Chlorella fusca* was linear with respect to time at least up to 90 min of incubation at 28°C (Fig. 1). The intercept of the *y* axis probably represents ethane[^{35}S]sulfonate nonspecifically adsorbed to the algal cell surface and was comparable to the adsorption rate shown by previous heat-killed algal cell.

(b) Cell density. Ethanesulfonate uptake was directly proportional to the cell density up to $3 \cdot 10^8$ cells/ml for the duration of the assay. Cell densities of $(1-2) \cdot 10^8$ cells/ml were used for all subsequent uptake studies.

Efflux rates

Efflux studies were conducted with cells exposed to ethane[^{35}S]sulfonate for one hour and resuspended afterwards in the incubation medium containing unlabelled ethanesulfonate. Negligible loss of counts from the cells were found over a 30 min period indicating little exchange of the internal ^{35}S-labelled ethanesulfonate with the external unlabelled compound, respectively, a rapid metabolization of the labelled ethanesulfonate within the alga.

Effect of pH and temperature on ethanesulfonate uptake

Both pH and temperature had pronounced effects on the specific rates of ethanesulfonate uptake in *Chlorella fusca* (Figs. 2 and 3).

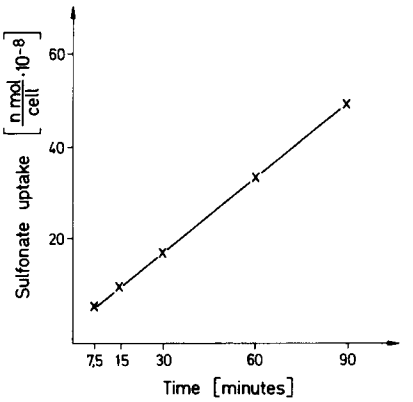


Fig. 1. Time dependence of ethanesulfonate uptake in *Chlorella fusca*. Algae were grown for 4–5 days on ethanesulfonate, washed intensively with S-free medium and resuspended to a density of 10^8 cells/ml in S-free medium. After addition of ethane[^{35}S]sulfonate, the Warburg vessels were incubated for the time intervals mentioned using the following conditions: 28°C, 4500 lux and a final concentration of 0.3 mM for ethanesulfonate.

A pH of 7.8 was found to be optimal using 0.01 M potassium phosphate as buffer. Since ethanesulfonate is a monovalent anion throughout the whole pH range tested, any effect of the external pH from 5 to 8.5 should be explained as effects on

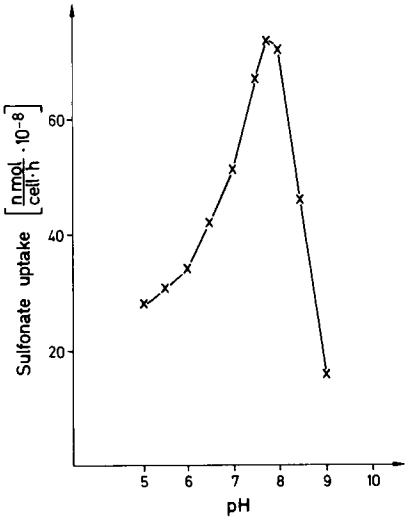


Fig. 2. pH dependence of ethanesulfonate uptake. Washed cells were incubated in 2 ml of 0.01 M KH_2PO_4/K_2HPO_4 buffer (pH 5–8.5) for 30 min. Principal conditions as described in Fig. 1.

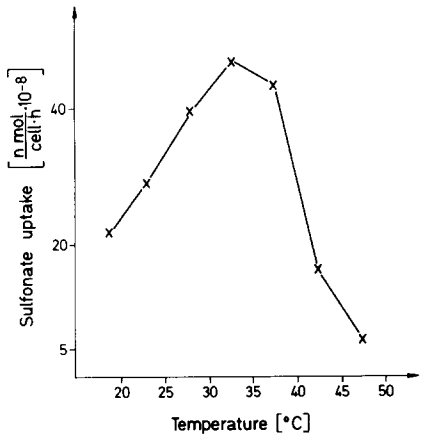


Fig. 3. Temperature dependence of ethanesulfonate uptake. Washed algae were suspended in the prewarmed medium and assayed for ethanesulfonate uptake during 15 min using the conditions described in Fig. 1.

the cell surface or the uptake system itself, e.g. upon protonation/deprotonation of the transport sites.

The temperature optimum of ethanesulfonate uptake was about 33°C (Fig. 3). At higher temperatures uptake of the heat-stable ethanesulfonic acid decreased rapidly, probably due to the inactivation of this uptake system. From the Arrhenius plot an activation energy of 41.3 kJ/mol between 18°C and 33°C was calculated (data not shown).

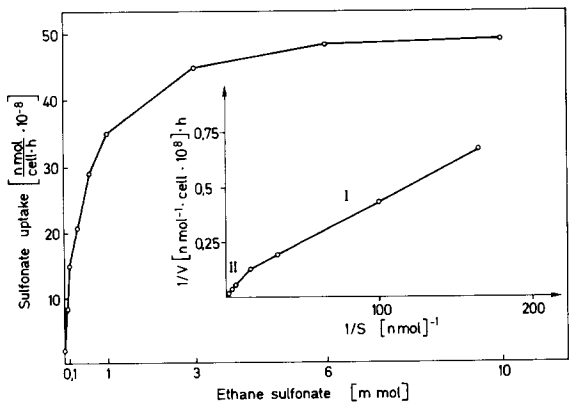


Fig. 4. Concentration dependence and Lineweaver-Burk plot of ethanesulfonate uptake. Conditions as in Fig. 1. Transport rates were determined from three samples taken within 15 min after adding the radiolabelled ethanesulfonic acid.

Concentration dependence of ethanesulfonate uptake

The rate of ethanesulfonate uptake was dependent on its concentration in the medium and conforms to Michaelis-Menten type saturation kinetics (Fig. 4). The double reciprocal Lineweaver-Burk plot showed a biphasic response over the concentration range from 10^{-6} to 10^{-2} M with K_m values in increasing order:

Phase I ($1 \cdot 10^{-6}$ M to $6 \cdot 10^{-5}$ M): $K_m = 5.0 \cdot 10^{-5}$ M

Phase II ($6 \cdot 10^{-5}$ M to $1 \cdot 10^{-2}$ M): $K_m = 3.8 \cdot 10^{-4}$ M

Substratespecificity of ethanesulfonate uptake

The specificity was tested by competition experiments using various sulfonates and propionate in a 10-fold concentration to ethanesulfonate (Table I). Obviously all sulfonic acids tested competed with ethanesulfonate uptake, independently of the different functional groups in the molecules. Neighboured HO-, NH_2-, HS-, HOOC-, or even benzylic groups with almost distinct charge or space geometry did not affect the recognition of the sulfonic group. Thus, the specificity of the ethanesulfonate uptake system in *Chlorella fusca* seems to be limited to the sulfonate group itself.

On the other hand the alga could clearly distinguish between sulfonate groups and carboxylic ones as the 'C-analogue' propionate failed to compete with ethanesulfonate indicating a new uptake system for sulfonic acids besides the monocarboxylate carrier.

The effect of other compounds and analogues

Ethanesulfonate uptake is furthermore inhibited by the anorganic 'sulfonate analogues' thiosulfate and tetrathionate (Table I), but not by any of the other common sulfur anions (sulfate, sulfite) or common sulfur amino acids (cyst(e)ine and methionine) added to the assay.

Surprisingly cyst(e)ine, methionine, and cysteic acid stimulated ethanesulfonate uptake slightly, similar to other amino acids. Obviously cysteic acid, whose sulfonate group did not interfere with ethanesulfonate uptake was solely recognized as an amino acid by *Chlorella fusca*, although it could be metabolized as sulfur source.

TABLE I

EFFECT OF STRUCTURAL ANALOGUES AND OTHER S-COMPOUNDS ON ETHANESULFONATE (ES) UPTAKE

Washed algae were preincubated for 3–5 minutes in the unlabelled and to 3 mM concentrated analogue before adding ^{35}S-labelled ethanesulfonate to the incubation medium. Principal conditions as described in Fig. 1. All compounds were used as sodium salts with exception of the amino acids.

Compound	ES uptake (% of control)
None	100
Methanesulfonate	21
Ethanesulfonate	24
Propanesulfonate	23
Butanesulfonate	22
Pentanesulfonate	20
2-Mercaptoethanesulfonate	24
2-Hydroxyethanesulfonate	33
2-Aminoethanesulfonate	31
Sulfoacetate	25
Benzenesulfonate	44
Propionate	104
Sulfate	102
Sulfite	101
Thiosulfate	81
Tetrathionate	65
Cysteine	140
Methionine	145
Cysteic acid	142
Alanine	140
Valine	144

TABLE II

EFFECT OF VARIOUS INHIBITORS ON ETHANESULFONATE (ES) UPTAKE IN *CHLORELLA FUSCA*

Washed algae were preincubated for 3–5 min in each compound before adding ^{35}S-labelled ethanesulfonate to the incubation medium. Principal conditions as described in Fig. 1. The uptake rates are corrected for non-specific adsorption. Abbreviations as defined. Substances marked with * are solubilized in ethanol.

Inhibitor	Concn. (mM)	ES uptake (% of control)
None		100
Sodium fluoride	1.0	115
Sodium iodoacetate	1.0	125
Sodium cyanide	1.0	96
Sodium azide	1.0	94
DCMU*	1.0	89
'Darkness'		98
Dibromothymochinon*	1.0	0
	0.1	35
	0.01	80
2,4-DNP*	1.0	0
CCCP*	1.0	0
	0.1	92
	0.01	112
DCCD*	1.0	38
	0.1	92
	0.01	112
NEM*	1.0	29
PCMBS	1.0	0
PMSF*	1.0	0
	0.1	63
	0.01	102
Ethanol (1%)	117 (!)	63

Effect of various inhibitors on ethanesulfonate uptake

A variety of metabolic inhibitors was tested for their effects on ethanesulfonate uptake (Table II). Ethanesulfonate uptake was not affected by inhibitors of glycolysis (iodoacetate and fluoride); it was little affected by some inhibitors of respiration and photosynthesis (azide, cyanide, DCMU, 'darkness'), whereas DBMIB inhibited markedly. Some uncouplers showed substantial inhibition (2,4-dinitrophenol and CCCP), especially the protonophore CCCP was the most effective metabolic inhibitor tested. The ATPase inhibitor DCCD was inhibitory as well.

On the other hand *N*-ethylmaleimide and *p*-chloromercuribenzylsulfonate (PCMBS), two sulfhydryl group reagents, caused extensive inhibition of ethanesulfonate uptake suggesting the involvement of SH-groups in the uptake process. Further specification was allowed by the strong inhibition of the non-penetrating reagent PCMBS reacting solely with sulfhydryl groups on the outer surface of the cell membrane showing the importance of such groups.

The effect of phenylmethylsulfonyl fluoride (PMSF) was also studied. This protein-modifying

reagent, known to block irreversibly active site serine hydroxyl groups by sulfonation [8], showed remarkable inhibition. Also ethanol was found to be inhibitory, an important fact as most of the inhibitors tested are solubilized in ethanol. Therefore 1% ethanol (0.17 M) was obligatory present in most of the assays and two control tests with/without ethanol each were made in all experiments.

Effect of other ions on ethanesulfonate uptake

Ethanesulfonate uptake in these assays was measured in a different system using deionized water-washed cells and a 0.001 M KH_2PO_4/KH_2PO_4 buffer (pH 7.8) containing various anions and cations at 3 mM concentrations. As shown in Table III none of the monovalent anions tested (F-, Cl-, NO_3^-, acetate, and propionate) affected ethanesulfonate uptake, whereas most of the metal ions stimulated uptake; only the heavy metals Hg^{2+} and Cu^{2+} inhibited significantly.

TABLE III

EFFECT OF VARIOUS ANIONS AND CATIONS ON ETHANESULFONATE (ES) UPTAKE

Washed algae were preincubated for 3–5 min in the 3 mM concentrated compound before adding labelled ethanesulfonate to 1 mM K_2HPO_4/KH_2PO_4 buffer (pH 7.8). Principal conditions as described in Fig. 1. Cations were used as the chlorides and anions as the sodium salts. Data are not corrected for a possible complexation with phosphate.

Anion/cation	ES uptake (% of control)
None	100
Fluoride	105
Chloride	103
Nitrate	100
Acetate	100
Propionate	104
NH_4^+	118
Li^+	111
Na^+	108
K^+	106
Mg^{2+}	234
Ca^{2+}	225
Mn^{2+}	250
Fe^{2+}	211
Zn^{2+}	222
Cu^{2+}	10
Hg^{2+}	2
Fe^{3+}	250
Al^{3+}	265

The failure of other monovalent anions to inhibit ethanesulfonate uptake confirmed the former assumption of an unique ethanesulfonate uptake system with separate binding sites for the transport of this 'nutrient' different from other anionic uptake systems in *Chlorella fusca*.

On the other hand most of the metal ions tested has pronounced effects on ethanesulfonate uptake. Although *Chlorella fusca* showed already acceptable uptake rates using 0.3 mM sodium ethanesulfonate in 1 mM phosphate buffer (pH 7.8), divalent cations (Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Zn^{2+}) and trivalent ions (Fe^{3+}, Al^{3+}) stimulated sulfonate uptake 2–3-fold. Identical effects were observed with all divalent and trivalent cations tested, whereas their anions were interchangeable. Monovalent cations (Li^+, Na^+, K^+, 'NH_4^+') were less effective in stimulating ethanesulfonate uptake but showed yet a positive effect in order of their ionic strength.

These results suggest to discuss the effect of cations on ethanesulfonate uptake via reduction of the surface potential rather than by an optimal ionic strength per se (inducing the proper conformational state of the uptake system?) or by specific effects of a single metal ion [4,11]. Similar results were presented for sulfate uptake in yeast [11], and the authors suggested consistently that "...multivalent cations might stimulate uptake of anions by reduction of the surface potential. This reduction will favour the accumulation of anions at the membrane-solution interface and thus enhance the rate of anion uptake." In *Chorella fusca* multivalent cations might therefore support unspecifically approach and binding of ethanesulfonate to its specific uptake system, although effects via membrane potential or cell pH may contribute to the observed effects of cations.

The effect of phosphate and related compounds on ethanesulfonate uptake

Phosphate is the most effective compound in stimulating sulfonate uptake independently of its corresponding cation. Fig. 5 shows ethanesulfonate uptake to be directly proportional to the

logarithmic plotted phosphate concentration from 1 mM to 100 mM. At higher concentrations ethanesulfonate uptake decreased rapidly due to the high extracellular osmotic strength. This response is specific for ethanesulfonate uptake in *Chlorella fusca*, since sulfate uptake is not affected by the phosphate concentration in the medium (Fig. 5). The 'phosphate effect' is also present, if algal cells were only preincubated in phosphate buffer. Even preincubation in 1 mM phosphate buffer stimulated ethanesulfonate uptake 5-fold compared to the normal assay. Obviously the phosphate-effect depends on the availability of phosphate ions inside the cell.

Consistently stimulation of ethanesulfonate uptake is not restricted to anorganic phosphate itself, but can also be shown by some 'phosphate-containing' compounds serving as donor of phosphate groups (phospho*enol*pyruvate, ATP, GTP, or CTP) as shown in Table IV. Compounds without energetisized phosphate groups such as 3-phosphoglyceric acid, glucose 6-phosphate, β-glycerophosphate, or AMP did not affect ethanesulfonate uptake. Aminoethyl phosphate, known to inhibit alkaline phosphatases in animal systems [5] stimulated ethanesulfonate uptake substantially, whereas the phosphate antagonist pyrophosphate inhibited markedly ethanesulfonate transport in *Chlorella fusca* assuming altogether regulation of the active state of ethanesulfonate uptake system by phosphorylation/dephosphorylation.

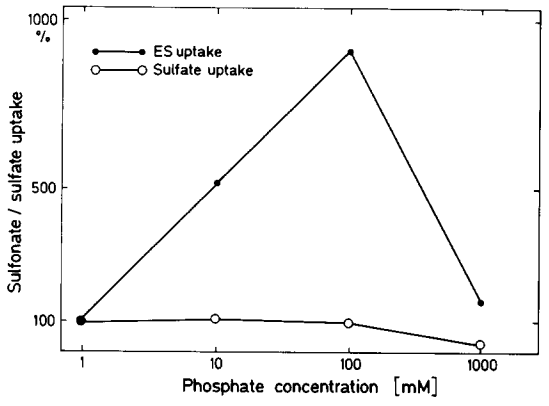


Fig. 5. Effect of phosphate concentration on ethanesulfonate and sulfate uptake. Washed algae were incubated immediately for 30 min in KH_2PO_4/K_2HPO_4 buffer pH 7.8 after adding 0.3 mM ^{35}S-labelled ethanesulfonate or sulfate to the assay. The phosphate concentration was varied as indicated. 100% uptake for sulfate (ethanesulfonate) are 620 (425) nmol·h^{-1}·cell^{-1}·10^{-9}.

TABLE IV

EFFECT OF PHOSPHATE AND PHOSPHORYLATED COMPOUNDS ON ETHANESULFONATE (ES) UPTAKE

Washed algae were preincubated for 3–5 min in each 3 mM concentrated compound before adding labelled ethanesulfonate to the incubation medium. Conditions as described in Fig. 1.

Compound	ES uptake (% of control)
None	100
Phosphate	270
Phospho*enol*pyruvate	155
CTP	158
GTP	149
ATP	161
ADP	127
AMP	104
3-Phosphoglyceric acid	102
Glucose-6-phosphate	101
β-Glycerophosphate	104
Aminoethyl phosphate	187
Pyrophosphate	48

Identity of radioactivity taken up

After incubation in 0.3 Mm ethane[^{35}S] sulfonate for one hour the incorporated radioactivity was further examined as described in Materials and Methods. The results of these experiments are shown in Table V: More than 96% of the radioactivity was taken up into a water-soluble form, 2% solubilized in the methanol-chloroform fraction and was later identified as sulfolipid, whereas less than 2% remained unsoluble.

The water-soluble radioactivity was subjected to chromatographic and electrophoretic analysis (Fig. 5). In both systems the majority of radioactivity did not co-chromatograph/co-electrophorete with authentic ethanesulfonate, indicating that most of the ethane[^{35}S]sulfonate taken up had been metabolized to other compounds. Nevertheless, in both systems a small peak moving to the position of ethane[^{35}S]sulfonate was detected, other peaks of the electrophoretic system behaved as glutathione, cyst(e)ine and methionine. The

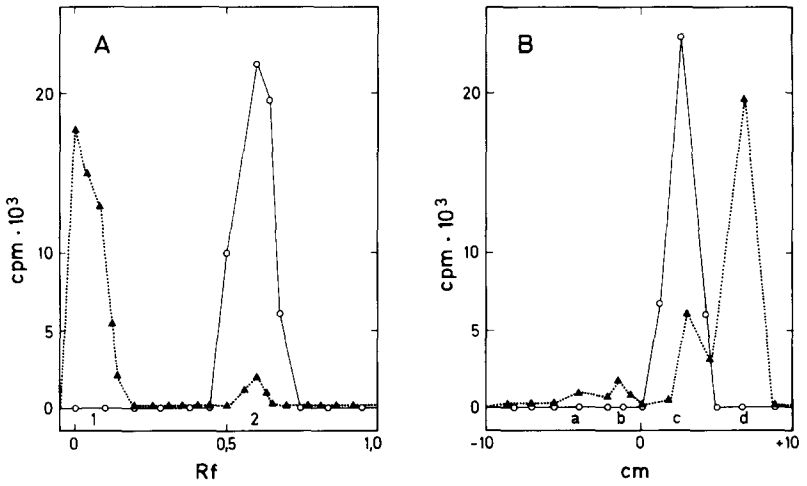


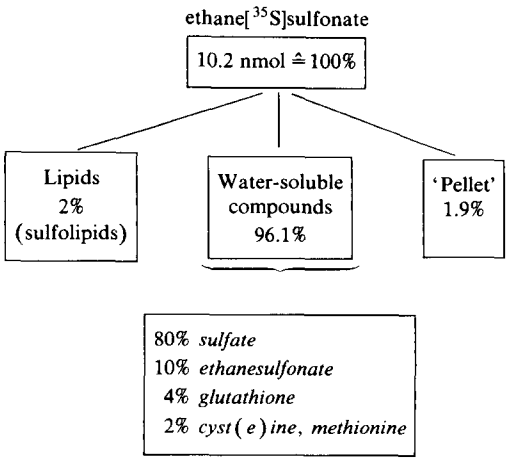
Fig. 6. Chromatographic (A) and electrophoretic (B) analysis of water-soluble radioactivity. This fraction was prepared and assayed as described in Materials and Methods. In all cases ▲------▲ represents the water-soluble radioactivity and ○ ——— ○ labelled ethanesulfonate. (A) TLC: solvent isopropanol/NH_3/H_2O (20:1:4, v/v). (B) TLE: solvent 0.1 M citrate buffer (pH 5.8) at 25 V/cm for 30–40 min.

main peak, however, containing 80% of the radioactivity taken up could be identified as anorganic sulfate.

TABLE V

IDENTIFICATION OF THE RADIOACTIVE PRODUCTS FORMED FROM ETHANE[^{35}S]SULFONATE TAKEN UP

Washed algae were incubated for 1 h in 0.3 mM labelled ethanesulfonate using a 10-fold higher specific activity than usual (650000 Bq/nmol). After this period the incorporated radioactivity was prepared and analyzed as described in Materials and Methods.



Discussion

The ethanesulfonate uptake system of *Chlorella fusca* qualifies as an active and carrier-mediated membrane transport system ('permease') for a number of reasons: (a) The transport system has a high affinity for its substrate, ethanesulfonate, and conforms to Michaelis-Menten saturation type kinetics, indicating a finite number of binding or entry sites for ethanesulfonate. (b) Ethanesulfonate transport is markedly temperature dependent, indicating ethanesulfonate not to penetrate by simple diffusion. (c) Ethanesulfonate transport is highly pH dependent, whereas ethanesulfonate represents a monovalent anion throughout the whole pH-range tested. (d) Ethanesulfonate uptake is an energy-requiring process as shown by the action of the metabolic inhibitors, 2,4-dinitrophenol, CCCP, DCCD, or DBMIB, preventing ethanesulfonate uptake substantially. The nature of its energy source however, requires further discussion, since azide, cyanide, DCMU, or 'darkness' failed largely to affect ethanesulfonate uptake in *Chlorella fusca*. Apparently only a small fraction of cellular energy (ATP) is involved in ethanesulfonate uptake and the remaining energy-dependent processes of photphosphorylation or oxidative phosphorylation are sufficient to

maintain ethanesulfonate uptake. (d) Ethanesulfonate uptake is unidirectional, i.e. the permease does not catalyze an exchange between internal and external substrate. (f) Uptake was inhibited by structural analogues. The uptake system is highly specific for the sulfonate group, neither monocarboxylates nor other monovalent anions interfere with ethanesulfonate uptake. (g) The uptake system is not constitutive, as sulfate-grown algae do not possess this ability, excluding non-specific permeation of the sulfonic acid [3]. As shown earlier, ethanesulfonate cannot be caused via substrate induction (the presence of ethanesulfonate in the medium), but develops solely and specifically in response to sulfate limitation. This development is inhibited by actidione, showing the involvement of de novo protein synthesis, and can be reverted within several hours by addition of external sulfate in vivo (unpublished results).

These results meet the requirement of an active transport process, mediated by an enzyme-like permease in *Chlorella fusca*. Ethanesulfonate uptake occurs through a distinct system and the existence of specific binding sites for ethanesulfonate during uptake is suggested by the effects of the structural analogues in contrast to the practical ineffectiveness of other monovalent anions (Cl-, NO_3^-, pronionate...) or divalent anions (SO_4^{2-}). Structural requirement for reaction with the ethanesulfonate transport system is only the sulfonate group itself, the system can neither distinguish between the structural analogues of ethanesulfonate nor shows preference for any of the sulfonates tested. It may be allowed to speculate whether this system represents a general sulfonic acid permease, as known for amino acid permeases by other organisms.

Most of the radioactivity taken up appeared as sulfate, probably the first degradaton product of ethanesulfonate in this alga. Besides sulfate other compounds of sulfur metabolism as sulfolipids, glutathione, cyst(e)ine, and methionine could also be detected. For that reason it was not possible to estimate whether ethanesulfonate uptake occurs against a concentration gradient. At this time we can only describe the entry of ethanesulfonate into *Chlorella fusca* via an active uptake system, that may involve both transport and metabolism as discussed for taurine uptake in *Staphylococcus aureus* M [1].

Ethanesulfonate uptake could be stimulated by phosphate. Apparently it was coupled to the availability of phosphate ions inside the cell and to phosphate-mediated metabolic processes. Phosphate stimulation of enzymes can be explained by several possibilities e.g., facilitation of enzyme phosphorylation by protein kinases or inhibition of phosphatases.

The key to further explanation gives the action of both aminoethylphosphate and pyrophosphate. Whereas the phosphatase inhibitor aminoethyl phosphate stimulated ethanesulfonate uptake markedly, the cellular phosphate antagonist, ATP-analogon and ATPase-inhibitor pyrophosphate decreased ethanesulfonate uptake strongly. Also two inhibitors of protein phosphorylation in animal systems, phenylmethylsulfonyl fluoride (PMSF) and ethanol [7,10] decreased ethanesulfonate uptake substantially. Especially PMSF, known to inhibit irreversibly active site serine hydroxyl groups as well as tyrosine sites where phosphorylation could take place, was strongly effective.

We propose that the activity of the ethanesulfonate permease may be regulated by a phosphorylation/dephosphorylation mechanisms involving either the permease itself of some closely related membrane proteins. According to this hypothesis, the uptake system might bind ethanesulfonate only in a phosphorylated status as described earlier for chloride transport by *Acetabularia* [9].

Furthermore this hypothesis may help to clarify the uptake mechanism of ethanesulfonate in *Chlorella fusca* as (a) a primary active transport by an ATP-dependent electrogenic pump (as shown for *Acetabularia* [9]) or (b) a 'chemiosmotic' secondary active transport linked to a proton gradient. Our data are at present not sufficient to enable a decisive choice between them, although several aspects may give a hint to possibility (a).

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