Sulphur Sources for Growth of *Chlorella fusca* and Their Influence on Key Enzymes of Sulphur Metabolism

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The green alga *Chlorella fusca* strain 211-8b (algal collection of Göttingen) is able to grow on more than 100 different sulphur sources such as mercaptides, disulphides, thioethers, sulphinic and sulphonic acids including 'Good buffers', sulphoxides, sulphones and sulphate esters. This suggests that green algae might be of importance for degradation of xenobiotics and natural compounds in the overall sulphur cycle. The data presented describe the growth of *C. fusca* on such sulphur compounds and the influence of these compounds on enzymes of the assimilatory sulphate reduction sequence.

INTRODUCTION

Sulphur compounds are formed, released and degraded in nature as normal constituents of the environment in a series of reactions summarized as the sulphur cycle (Zehnder & Zinder, 1980). However, man-made sulphur compounds in the form of volatiles such as SO₂ and H₂S or bound forms of sulphur such as catalysts, tensides, organic solvents and drugs are discharged in increasing amounts to our environment (Kellog *et al.*, 1972). These compounds cause severe trouble not only to plants (SO₂ toxicity, acid rain), but possibly also to the aquatic system. The effect of anthropogenic sulphur compounds on green algae has not been studied systematically, although a survey using the cyanobacterium *Synechococcus* (*Anacystis nidulans*) has appeared (Schmidt *et al.*, 1982). Only limited information is available about sulphur-containing substances used for growth of higher plants (Brunold & Erismann, 1975; Ellis, 1969; Hart & Filner, 1969), algae (Krauss, 1984; Saito, 1976) and cyanobacteria (Schmidt *et al.*, 1982). Furthermore these substances could interfere with metabolic pathways such as uptake systems or regulatory signals of sulphur and nitrogen assimilation (Reuveny & Filner, 1977; Zink, 1984). This study was initiated to obtain some information on the possible value of a green alga for the detoxification of sulphur compounds of either biotic or abiotic origin.

METHODS

Organism. *Chlorella fusca* strain 211-8b of the algal collection of Göttingen, FRG, was cultured on a sulphur-deficient medium (SD medium). It contained (μM): KH₂PO₄, 500; Mg(NO₃)₂·6H₂O, 2000; KNO₃, 5000; K₂HPO₄, 500; Ca(NO₃)₂, 250; H₃PO₄, 23; Na₂MoO₄·2H₂O, 44; Mn(NO₃)₂·6H₂O, 6·3; ZnCl₂, 0·37; Cu(NO₃)₂·H₂O, 0·16; plus 1 ml l⁻¹ of Fe-EDTA complex (8·9 g FeCl₂·4H₂O and 16·7 g EDTA·2H₂O dissolved in 500 ml water and aerated overnight; the filtrate was used) and 1 ml l⁻¹ of trace elements (containing, per litre, 1·43 mg H₃BO₃, 10 mg NaMoO₄·2H₂O, 50 mg ZnCl₂ and 38 mg Cu(NO₃)₂·2H₂O).

The sulphur compounds of Tables 1–4 were added to a final concentration of 0·3 mM whereas the sulphur control contained 0·2 mM Na₂SO₄. The preparation and addition of the sulphur sources is described below. Pirson flasks containing 750 ml growth medium were inoculated with 6×10^6 cells, equivalent to about 0·002 mg chlorophyll. Cultures were grown in a Kniese apparatus at 27 °C and 14000 lx using normal air as CO₂ source.

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Abbreviations: APS, adenosine 5'-phosphosulphate; DTE, dithioerythritol.

Algal flasks were sterilized and only axenic cultures of *C. fusca* strain 211-8b were used. Purity of cultures was checked by plating. When sulphur sources were added as solids after autoclaving, sterility could not be guaranteed; however, no evidence of contamination was evident by microscopical analysis.

Preparation of crude extracts. All cultures were harvested after 5 d growth (120 h). Cells were centrifuged for 15 min at 3000 g, washed once with 0.1 M-Tris/HCl buffer pH 8.0 containing 10 mM-MgCl₂ and 10 mM-mercaptoethanol and taken up in 5 ml of the same buffer per g wet weight of cells. A 300 µl sample was removed for chlorophyll determination, and the remaining cells were broken in a French press at 12000 lbf in⁻² (~84000 kPa). This crude extract was cleared by centrifugation for 10 min at 10000 g. The supernatant was used for the enzyme tests.

Chlorophyll determination. The 300 µl sample of resuspended cells (see above) was extracted twice with 5 ml methanol at 80 °C for 5 min. The extracted chlorophyll was determined according to Arnon (1949).

Determination of the sulphate activation capacity. The enzymes of the sulphate activation sequence, ATP-sulphurylase (EC 2.7.7.4) and adenosine 5'-phosphosulphate- (APS-) sulphotransferase, were assayed according to Schmidt (1972). Assay mixtures contained 100 µmol Tris/HCl pH 8.5, 10 µmol MgCl₂, 6 µmol ATP, 10 µmol dithioerythritol (DTE), 2 µmol Na₂³⁵SO₄ (65 kBq nmol⁻¹) and enzyme (usually 100 µl) in a total volume of 1 ml, and were incubated at 37 °C for 1 h under N₂. For determination of labelled sulphite formed, 100 µmol non-labelled sulphite was added and acid-volatile radioactivity was trapped in 1 ml 1 M-triethanolamine using Conway discs. Radioactivity was determined in a Beckman LS-100 scintillation counter using the scintillation cocktail Rotiscint 22 (Roth).

Other enzyme assays. Cysteine synthase (EC 4.2.99.8) was determined according to Schmidt (1977). Assay mixtures contained 100 µmol Tris/HCl pH 7.5, 3 µmol DTE, 5 µmol Na₂S, 5 µmol *O*-acetyl-L-serine and enzyme (usually 10 µl of crude extract) in a total volume of 1 ml. The reaction was started by addition of *O*-acetyl-L-serine and incubation was continued for 30 min at 37 °C. Cysteine was determined as the red ninhydrin reaction product formed under acidic conditions (Gaitonde, 1967) modified as follows. To 1 ml of the test material 1 ml Gaitonde reagent (1.25 g ninhydrin dissolved in 20 ml conc. HCl and 80 ml conc. acetic acid) was added and the mixture was incubated for 10 min in a boiling water bath. After cooling in an ice bath for 5 min, 2 ml *n*-butanol was added and mixed to stabilize the complex. Blanks were run without *O*-acetyl-L-serine. The absorption maximum of this red reaction product is at 560 nm; for our determination, however, we used a Zeiss filter photometer at 546 nm. The absorption coefficient for 546 nm was determined as 10.1 × 10⁶ l mol⁻¹ cm⁻¹. It should be noted, however, that this absorption coefficient varies with age of the acidic ninhydrin reagent (Krauss, 1984).

The activities of D- and L-cysteine desulphydrase were determined by production of H₂S from either D- or L-cysteine using the methylene blue method (Siegel, 1965). For L-cysteine desulphydrase activity (EC 4.4.1.1) the assay mixtures contained 100 µmol Tris (free base), 2 µmol DTE, 800 nmol L-cysteine and enzyme (usually 100 µl) in a total volume of 1 ml. The reaction was started by addition of L-cysteine. The test tubes were closed with rubber caps and incubated at 37 °C for 30 min. Sulphide formed was determined after addition of 0.1 ml 0.2 M-dimethyl-*p*-phenylenediamine dissolved in 7.2 M-HCl and 0.1 ml 0.3 M-FeCl₃ dissolved in 1.2 M-HCl. Methylene blue was determined at 670 nm in a Beckman DU-7 spectrophotometer against a blank without addition of enzyme. For correction for internal cysteine a control was run using crude extract without addition of L-cysteine. A molar absorption coefficient of 28.5 × 10⁶ l mol⁻¹ cm⁻¹ was used for methylene blue at 670 nm. For D-cysteine desulphydrase activity the assay mixtures contained 100 µmol Tris/HCl pH 9.0, 2 µmol DTE, 800 nmol D-cysteine and enzyme (usually 100 µl) in a total volume of 1 ml. Blanks, incubation conditions and methylene blue determination were as described above for L-cysteine desulphydrase activity.

Thiosulphate reductase activity was determined as sulphide production from thiosulphate with DTE as electron donor (Schmidt *et al.*, 1984). Assay mixtures contained 100 µmol Tris/HCl pH 9.0, 2 µmol DTE, 200 nmol thiosulphate and enzyme (usually 100 µl) in a total volume of 1 ml. The test was started by addition of enzyme and incubated for 30 min at 37 °C. Sulphide formed was determined as described earlier (Schmidt *et al.*, 1984) as methylene blue. Blanks without substrate or enzyme were subtracted. A molar absorption coefficient at 670 nm of 12.01 × 10⁶ l mol⁻¹ cm⁻¹ was used. It should be noted that higher thiosulphate concentrations inhibit methylene blue formation.

Sulphur sources. Sulphur compounds were obtained from the sources indicated in Tables 1–4. Abbreviations: B, Boehringer Mannheim; E, EGA-Chemie; F, Fluka; H, Riedel-de-Haen; K, synthesized by Dr H. P. Köst (this Institute); M, Merck; P, Pierce Eurochemie; R, Roth; S, Sigma; U, Unfried; V, Serva.

SD medium was supplemented with 0.3 mM sulphur-containing compound, since higher concentrations could be toxic (e.g. L-cysteine inhibited growth above a concentration of 0.5 mM). All water-soluble compounds were added after sterile filtration. Insoluble compounds, as indicated in Tables 1–4, were added as solids after autoclaving of the medium; in these cases the final concentration in the medium depends on the solubility and therefore is not exactly known. All substances were analysed for sulphate contamination by using barium acetate. If barium sulphate was detected, it was removed by centrifugation. Excess barium was removed by treatment with a Dowex-50 WX8 (100–200 mesh, H⁺ form) resin. The chemicals used were thus free of inorganic sulphate as judged by this method.

Table 1. Influence of inorganic sulphur compounds on growth and enzyme activities of *C. fusca*

The following key also applies to Tables 2–4. *m*, mean, σ , standard deviation. A, Addition to SD medium (–, without sulphate; +, with sulphate); C, company supplying the sulphur source (see Methods); T, treatment of the sulphur source (s, addition as solid after autoclaving; f, free of sulphate by the Ba²⁺ method). Chl., Chlorophyll content (mg per 750 ml medium) (* denotes a high starch content, indicated by a white ring, identified as starch by the I₂/KI reaction, in the crude extract pellet); SA, sulphate activation sequence [nmol acid-volatile radioactivity (i.e. ³⁵SO₄^{2–}) (mg chlorophyll)^{–1} h^{–1}]; CS, cysteine synthase [μ mol cysteine (mg chlorophyll)^{–1} h^{–1}]; L-CD, L-cysteine desulphhydrase [μ mol sulphide (mg chlorophyll)^{–1} h^{–1}]; D-CD, D-cysteine desulphhydrase [μ mol sulphide (mg chlorophyll)^{–1} h^{–1}]; TSR, thiosulphate reductase [μ mol sulphide (mg chlorophyll)^{–1} h^{–1}]. ND, Not determined; NG, no growth.

Sulphur source		A	C	T	Chl.	SA	CS	L-CD	D-CD	TSR
Sodium sulphate (<i>n</i> = 93)	<i>m</i>				3.81	884	124	0.53	1.59	5.5
	σ				1.32	444	57	0.23	0.43	1.2
	<i>m</i> + σ				5.13	1278	181	0.79	2.02	6.7
	<i>m</i> – σ				2.49	390	67	0.28	1.15	4.3
Sulphur deficiency		–			0.04	349	644	4.87	1.65	38.9
Elemental sulphur		–	U	s	0.65	409	371	0.17	2.30	176.6
		+			3.26	1060	200	0.24	1.56	6.5
Ferrous sulphide FeS		–	M	s	0.09	853	993	9.83	2.17	24.6
		+			4.68	741	82	ND	ND	ND
Sodium sulphate Na ₂ SO ₄ ·10H ₂ O		+	M		3.81	834	124	0.53	1.59	0.5
Sodium thiosulphate Na ₂ S ₂ O ₃		–	M		2.49	743	197	0.51	1.80	6.3
		+			3.53	980	232	0.45	1.81	5.6
Sodium tetrathionate Na ₂ S ₄ O ₆ ·2H ₂ O		–	M	s	2.70	1038	172	0.58	1.80	6.3
Sodium thiocyanate		–	M		0.29	1771	97	1.99	0.79	17.8

Chemicals. In addition to chemicals mentioned in Tables 1–4, Na₂³⁵SO₄ was obtained from Buchler, ADP and ATP from Boehringer Mannheim, glutamine and *O*-acetyl-L-serine from Sigma, Rotiscint 22 and methanol from Roth, and Dowex-50 WX8 resin, dimethyl-*p*-phenylenediamine and Tris buffer from Serva. All other chemicals not specifically mentioned were obtained from Merck. Chemical names and formulae are given according to the catalogue of Fluka.

RESULTS

General remarks

C. fusca strain 211-8b was cultured in SD medium supplemented with each sulphur compound to be tested. A control culture with sodium sulphate was always run. To guarantee reproducibility, each compound was tested three to five times.

The following enzyme activities were determined in the crude extract: the sulphate activation capacity (the sum of ATP-sulphurylase and APS-sulphotransferase), cysteine synthase, L- and D-isomer-specific cysteine desulphhydrases (for possible cysteine catabolism), and thiosulphate reductase (whose function is so far unknown). All specific activities listed are normalized to chlorophyll and not to protein since the sulphur compounds interfered with the protein assay. Enzyme activities within the standard deviation of the sulphate reference were regarded as normal activities; values outside this range are specifically mentioned. It should be noted that an increase or a decrease of the specific activities does not distinguish between an effect of the sulphur source at the level of gene regulation and an interaction with the enzyme itself.

High specific activities in conjunction with low chlorophyll concentrations have to be interpreted carefully, because comparatively low absolute enzyme activities may appear as high specific activities due to an extremely low chlorophyll content. Therefore cultures with less than 0.02 mg chlorophyll after 5 d growth on the test compound were defined as 'no growth'. Results obtained under sulphur starvation conditions are also shown (in Table 1), to demonstrate the

Table 2. *Influence of mercaptides and disulphides on growth and enzyme activities of C. fusca*

See Table 1 for key to abbreviations.

Sulphur source	A	C	T	Chl.	SA	CS	L-CD	D-CD	TSR
Ethanethiol	—			0.06	296	569	7.36	0.47	19.5
C ₂ H ₅ SH	+			2.10	701	186	0.47	1.92	5.1
2-Mercaptoethanol	—	M		3.95	605	215	0.70	1.82	8.2
HSCH ₂ CH ₂ OH	+			5.21	1270	170	0.42	2.45	5.4
2-Mercaptoethanesulphonic acid, HSCH ₂ CH ₂ SO ₃ H	—	P		2.49*	1525	380	1.28	2.72	12.0
	+			3.79	1194	228	0.52	1.46	7.9
3-Mercaptopropionic acid	—	M		0.26	176	626	2.51	3.04	18.9
HSCH ₂ CH ₂ CHOOH	+			1.72	357	266	1.36	1.36	6.5
β-Mercaptopyruvate	—	F		2.87	1438	131	0.48	1.72	4.4
HSCH ₂ COCOOH	+			2.14	1287	139	0.37	1.83	4.7
Thioacetic acid	—	F		NG	—	—	—	—	—
2,3-Dimercapto-1-propanol	—	M		NG	—	—	—	—	—
(BAL), HOCH ₂ CH((SH)CH ₂ SH	+			NG	—	—	—	—	—
Glutathione reduced	—	M		2.25*	550	252	0.82	1.76	10.1
C ₁₀ H ₁₆ N ₃ O ₆ SH	+			2.55	525	187	0.59	1.57	9.0
Glutathione oxidized	—	B		0.54	985	430	0.73	1.22	7.2
C ₂₀ H ₃₂ N ₆ O ₁₂ S ₂	+			6.64	763	68	ND	ND	ND
L-Cysteine	—	M		4.53*	1139	120	0.54	1.36	6.1
HSCH ₂ CH(NH ₂)COOH	+			5.16	855	107	0.46	1.86	5.4
D-Cysteine	—	M		3.14	514	227	0.25	1.33	9.8
HSCH ₂ CH(NH ₂)COOH	+			4.56	1324	137	0.51	2.20	7.9
N-Acetyl-L-cysteine	—	V		1.55*	209	242	1.42	1.57	11.6
HSCH ₂ CH(NHCOCH ₃)COOH	+			4.26	1014	181	0.37	1.82	6.2
L-Cysteine methyl ester	—	S		2.00*	1930	140	0.03	1.81	9.1
HSCH ₂ CH(NH ₂)COOCH ₃	+			2.56	1494	105	0.13	2.16	8.7
DL-Homocysteine	—	S	s	1.10*	1129	273	0.78	1.94	10.6
HSCH ₂ CH ₂ CH(NH ₂)COOH	+			2.05	766	205	0.57	1.82	6.2
Cysteamine	—	S		1.45*	902	347	1.26	2.96	18.3
NH ₂ CH ₂ CH ₂ SH	+			2.63	2212	210	0.03	1.82	10.9
DL-6,8-Thioctic acid amide (lipoic acid)	—	V	s	2.54	1636	129	0.43	2.23	13.0
HS(CH ₂) ₂ CH(SH)(CH ₂) ₄ CONH ₂	+			2.93	1508	130	0.66	1.23	5.1

drastic influence of sulphur limitation on growth and enzyme activities. In view of these dramatic effects it seemed advisable to use sulphate controls and sulphate-limited cultures as references for comparison. Sulphur starvation is characterized by low growth and high specific activities of enzymes used for sulphur metabolism. In parallel experiments the symptoms of sulphur starvation were suppressed by adding sulphate together with each sulphur source tested in order to characterize specific effects on growth and cell metabolism. Significant differences in specific activities – even in the presence of sulphate – are indications of a metabolic interaction.

Results for 55 sulphur sources are shown in Tables 1–4. Only results for compounds of biological occurrence, or those which are necessary to illustrate particular effects, are listed: tables giving results for all 120 substances tested have been deposited at the British Library Lending Division, Boston Spa, LS23 7BQ, UK, as Supplementary Publication no. SUP 28024 (11 pp.). Copies may also be obtained from the authors.

Influence of inorganic sulphur compounds

The growth of *C. fusca* on inorganic sulphur-containing substances and their influence on enzyme activities is shown in Table 1. Cultures grown on sodium sulphate were used as reference cultures; these grew well as judged by their chlorophyll content. Cysteine synthase normally had higher specific activities than the enzymes of the sulphate activation sequence, and D-cysteine desulphydrase had a higher specific activity than the enzyme specific for L-cysteine. Cultures grown under sulphate starvation were characterized by high activities of enzymes related to

Table 3. Influence of thioethers, sulphones, sulphoxides and other sulphur-containing compounds on growth and enzyme activities of *C. fusca*

See Table 1 for key to abbreviations.

Sulphur source	A	C	T	Chl.	SA	CS	L-CD	D-CD	TSR
α -Keto- γ -methylthiobutyrate	—	S		3.82	360	243	0.51	1.79	5.8
$\text{CH}_3\text{S}(\text{CH}_2)_2\text{COCOOH}$	+			3.16	1055	85	0.82	2.97	3.2
Dimethylsulphide (CH_3) ₂ S	—	M		0.15	1378	227	3.14	1.58	28.6
Dimethylsulphoxide (DMSO)	—	M		0.12	446	207	3.18	1.34	44.3
(CH_3) ₂ SO	+			6.37	870	117	0.27	1.12	23.8
S-Methyl-L-cysteine	—	V		3.07	983	262	0.81	0.97	10.8
$\text{CH}_3\text{SCH}_2\text{CH}(\text{NH}_2)\text{COOH}$	+			2.92	1125	380	0.51	1.09	8.2
S-Carbamoyl-L-cysteine	—	F		6.60	778	129	0.38	1.34	5.8
$\text{NH}_2\text{COSCH}_2\text{CH}(\text{NH}_2)\text{COOH}$									
L-Methionine	—	M		2.81	1175	145	0.89	2.04	8.6
$\text{CH}_3\text{S}(\text{CH}_2)_2\text{CH}(\text{NH}_2)\text{COOH}$	+			2.49	1719	163	0.30	1.44	5.0
D-Methionine	—	F		0.61	2018	281	1.92	2.04	16.4
$\text{CH}_3(\text{CH}_2)_2\text{CH}(\text{NH}_2)\text{COOH}$	+			1.55	1642	154	0.63	1.59	6.2
S-Methyl-DL-methionine	—	S		0.91*	3681	647	ND	ND	16.5
(CH_3) ₂ S(CH_2) ₂ CH(NH_2)COOH	+			5.15	609	105	ND	ND	6.0
DL-Ethionine	—	S		NG	—	—	—	—	—
$\text{CH}_3\text{CH}_2\text{S}(\text{CH}_2)_2\text{CH}(\text{NH}_2)\text{COOH}$	+			NG	—	—	—	—	—
L-Methionine sulfoximine	—	S		0.05	1201	238	2.53	2.53	19.1
$\text{CH}_3\text{SONH}(\text{CH}_2)_2\text{CH}(\text{NH}_2)\text{COOH}$	+			0.20	2933	184	ND	ND	ND
L-Methionine sulphone	—	S	s	1.33*	2953	152	ND	ND	28.0
$\text{CH}_3\text{SO}_2(\text{CH}_2)_2\text{CH}(\text{NH}_2)\text{COOH}$	+			2.30	1439	153	ND	ND	6.2
L-Thiazolidine-4-carboxylic acid	—	F		2.16	1052	194	0.61	2.23	9.5
$\text{CH}_2\text{SCH}_2\text{NHCOOH}$									
Sulphanilamide	—	V	s	NG	—	—	—	—	—
$\text{NH}_2\text{C}_6\text{H}_5\text{SO}_2\text{NH}_2$	+			NG	—	—	—	—	—
Sodium lauryl sulphate	—	V		0.60	2876	298	ND	ND	14.6
(SDS), $\text{CH}_3(\text{CH}_2)_{11}\text{OSO}_3\text{H}$	+			1.17	1588	166	ND	ND	ND
DL-Homocysteine thiolactone	—	V		NG	—	—	—	—	—
$\text{S}(\text{CH}_2)_2\text{CH}(\text{NH}_2)\text{CO}$									
Thiourea	—	S		0.31*	1958	123	ND	ND	9.9
(NH_2) ₂ S									

normal sulphur metabolism, except for the sulphate activation sequence, which had a lower specific activity after 5 d growth under sulphur starvation. A kinetic study revealed, however, that this sequence was first activated but decreased drastically after 5 d growth in SD medium when the sulphur stress became severe (Krauss, 1984).

Reduced inorganic sulphur compounds proved to be poor sulphur sources for growth; the enzyme activities were similar to those found under sulphur starvation conditions. Growth on oxidized sulphur compounds such as thiosulphate and tetrathionate was comparable to growth on sulphate. Elemental sulphur caused an increase in the activities of cysteine synthase and thiosulphate reductase.

Influence of mercaptides and disulphides

The influence of mercaptides and disulphides on growth and enzyme activities of *C. fusca* is shown in Table 2. Dimercaptopropanol (BAL) inhibited growth. Typical sulphur starvation effects were observed with alkylthiols such as ethanethiol and ethanedithiol, thioglycerol, *N*-acetyl-L-cysteine and thiosalicylic acid as sole sulphur source. A decrease in activity of the sulphate activation sequence was observed during growth on *N*-acetyl-L-cysteine and cystathionine whereas higher activities for this sequence were found during growth on β -mercaptopyruvate and cyst(e)ine esters. High cysteine synthase activity was found for

Table 4. *Influence of sulphinic and sulphonic acids on growth and enzyme activities of C. fusca*

See Table 1 for key to abbreviations.

Sulphur source	A	C	T	Chl.	SA	CS	L-CD	D-CD	TSR
Sulphamic acid $\text{NH}_2\text{SO}_3\text{H}$	—	V		0.15	1724	476	ND	ND	3.4
Ethanesulphonic acid $\text{CH}_3\text{CH}_2\text{SO}_3\text{H}$	+	M	f	4.71	905	59	ND	ND	ND
1,2-Ethanedisulphonate $\text{HO}_3\text{SCH}_2\text{CH}_2\text{SO}_3\text{H}$	—	S		3.15*	1249	212	ND	ND	11.5
Aminomethanesulphonic acid, $\text{NH}_2\text{CH}_2\text{SO}_3\text{H}$	+			4.59	1130	115	ND	ND	5.7
Taurine $\text{NH}_2(\text{CH}_2)_2\text{SO}_3\text{H}$	—	S		0.84*	1861	176	0.83	2.13	9.6
2-Mercaptoethanesulphonic acid, $\text{HSCH}_2\text{CH}_2\text{SO}_3\text{H}$	—	F		NG	—	—	—	—	—
Isethionic acid $\text{HOCH}_2\text{CH}_2\text{SO}_3\text{H}$	+			0.87	2181	62	ND	ND	ND
Sulphoacetic acid $\text{HOOCCH}_2\text{SO}_3\text{H}$	—	S		4.46	1302	96	0.60	2.16	6.1
L-Cysteic acid $\text{HO}_3\text{SCH}_2\text{CH}(\text{NH}_2)\text{COOH}$	+			4.36	835	124	0.62	1.45	5.5
Ligninsulphonic acid†	—	P		2.49*	1525	380	1.28	2.72	12.0
Benzenesulphonic acid $\text{C}_6\text{H}_5\text{SO}_3\text{H}$	+			3.79	1194	228	0.52	1.46	7.9
Phenol-4-sulphonic acid $\text{HOC}_6\text{H}_4\text{SO}_3\text{H}$	—	M	f	3.61	1356	179	ND	ND	8.0
Toluene-4-sulphonic acid $\text{CH}_3\text{C}_6\text{H}_4\text{SO}_3\text{H} \cdot \text{H}_2\text{O}$	+			3.42	1324	182	ND	ND	5.1
Sulphobetaine† $\text{CH}_3(\text{CH}_2)_{11}\text{N}(\text{CH}_3)_2(\text{CH}_2)_3\text{SO}_3\text{H}$	—	K	f	5.42	787	187	5.22	1.79	6.5
MES†	+			3.10	1301	112	0.63	2.09	5.1
HEPES†	—	V		4.19*	799	155	0.71	2.58	7.7
HEPSO†	+			3.87	974	94	0.58	2.09	4.9
	—	F		2.81*	1327	241	0.69	1.75	6.5
	+			3.23	955	153	0.39	1.66	5.3
	—	F		0.19	2377	639	4.44	2.71	20.4
	—	F		1.64*	2148	351	1.71	3.72	19.6
	—	M		0.43	107	233	1.97	3.80	15.7
	—	V		NG	—	—	—	—	—
	—	S		0.34	1189	598	0.78	1.98	6.9
	—	V		0.87*	3013	275	0.95	2.59	7.1
	—	V		1.47	290	289	1.22	4.24	11.1

† Ligninsulphonic acid, a mixture of ligninsulphonic acids with different degrees of polymerization; sulphobetaine, *N*-dodecyl-*N,N'*-dimethyl-3-ammonio-propanesulphonic acid; MES, 2-morpholinoethanesulphonic acid ($\text{CH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{NCH}_2\text{CH}_2\text{SO}_3\text{H}$); HEPES, 4-(2-hydroxyethyl)piperazine-1-ethane-2-sulphonic acid ($\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2\text{SO}_3\text{H})\text{CH}_2\text{CH}_2\text{NCH}_2\text{CH}_2\text{OH}$); HEPSO, 4-(2-hydroxyethyl)piperazine-1,2-hydroxypropane-3-sulphonic acid ($\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2\text{SO}_3\text{H})\text{CH}_2\text{CH}_2\text{NCH}_2\text{CH}(\text{OH})\text{CH}_2\text{OH}$).

alkylthiols, mercaptoethanesulphonic acid, thioglycolic acid, DTE, reduced glutathione, L-homocysteine and L-homocystine. L-Cysteine desulphydrase activity was increased only by mercaptopropionic acid and decreased by L-cysteine methyl ester and L-cystine diethyl ester. D-Cysteine desulphydrase was activated during growth on L-homocystine. Thiosulphate reductase had a higher activity during growth on reduced glutathione, D-cysteine, cyst(e)ine esters, L-homocystine, cysteamine and cystathionine.

Influence of thioethers, sulphones, sulphoxides and miscellaneous other sulphur-containing compounds

Results obtained with these sulphur-containing compounds are summarized in Table 3. Growth was inhibited by ethionine, methional, sulphanilamide, homocysteine thiolactone and sulphathiazole as a sulphur source in both presence and absence of sulphate. Typical effects on sulphur starvation were observed during growth on dimethyl sulphide, dimethyl sulphoxide (DMSO), thiodiglycolic acid, L-methioninol, *S*-methyl-L-methionine, djencolic acid, thiourea, thiamin, and benzenesulphonamide. An increase in activity of the sulphate activation sequence

was found during growth on dimethyl sulphoxide, L- and D-methionine, L-methioninol, L-methionine sulphoxide and the sulphate esters 2-aminoethyl hydrogensulphate and SDS. Cysteine synthase activity was increased by S-methyl- and S-benzyl-L-cysteine and L-methionine sulphoxide. Growth on biotin increased L-cysteine desulphydrase activity. Thiosulfate reductase activity was increased during growth on dimethyl sulphoxide, S-methyl-L-cysteine and 2-aminoethyl hydrogensulphate.

Influence of sulphinic and sulphonic acids

Results obtained for growth and enzyme activities with sulphonic acids as a sulphur source for growth are shown in Table 4. No growth was observed with aminomethanesulphonic acid or sulphobetaine. Slow growth with symptoms of sulphur deficiency was observed with methanesulphonic acid, 1,2-ethane disulphonate, glutathionesulphonic acid, benzene sulphinic and sulphonic acids and toluenesulphinic acid, orthanilic acid and 'Good buffers' such as DIPSO, MOPS, HEPES, and HEPESO. An increase in the specific activity of the sulphate activation sequence was noted for growth on isethionic acid, formamidinesulphinic acid and L-cysteinesulphinic acid. High activities of cysteine synthase were found during growth on sulphanilic acid whereas growth on formamidinesulphinic acid yielded high activities of both cysteine synthase and thiosulphate reductase. L-Cysteinesulphinic acid, sulphanilic acid and S-sulphosalicylic acid increased the activity of D-cysteine desulphydrase activity. No decrease in the activity of the enzymes analysed was observed during growth on sulphate together with sulphinic or sulphonic acids.

DISCUSSION

The sulphur-containing substances used in this study were selected for various reasons. (a) Biogenic oxidized and reduced sulphur compounds were analysed for their ability to support algal growth, to study possible regulatory influences of these compounds on the assimilatory sulphate reduction pathway. (b) Synthetic sulphur-containing chemicals (xenobiotics) were selected to study possible biodegradation mechanisms of green algae using *C. fusca* strain 211-8b. (c) The cyanobacterium *Synechococcus* 6301 could only use a limited spectrum of sulphur-containing substances for growth (Schmidt *et al.*, 1982); thus it was of interest to see whether the eukaryotic alga *C. fusca* could grow on the same sulphur sources.

Growth characteristics. It was surprising that *C. fusca* was able to use such a variety of sulphur compounds for growth, in contrast to the limited spectrum of sulphur compounds on which the cyanobacterium *Synechococcus* 6301 grew (Schmidt *et al.*, 1982). In contrast to our strain of *C. fusca*, *C. vulgaris* showed normal growth only on thiosulphate (0.2 mM; Hodson *et al.*, 1968), whereas L-cysteine, L-cystine, S-sulfo-L-cysteine, L-methionine and reduced glutathione (each 0.2 mM; Hodson *et al.*, 1971) caused reduced growth, and lanthionine, L-homocystine and L-cysteic acid inhibited growth (0.2 mM; Hodson *et al.*, 1968). L-Cysteine and glutathione inhibit the growth of tobacco cells (Rennenberg, 1981; Rennenberg & Uthemann, 1980); this may be explained by a shift in the ratio of reduced to oxidized thiols, thus causing regulatory problems (Krämer & Schmidt, 1984). Growth of the aquatic higher plant *Lemna* was inhibited by sulphide, reduced glutathione, L-cystine and L-methionine (each 4.7 mM; Ellis, 1969); this may have been due to the high concentrations used.

No growth was observed for *C. fusca* with the mercaptides 2,3-dimercaptopropanol (BAL), thioacetic acid and thiocresol, the thioethers ethionine, methional, sulphonamide, homocysteine thiolactone, and sulphathiazole, and the sulphonic acids aminomethanesulphonic acid and sulphobetaine. Some of these compounds, e.g. as BAL, ethionine and sulphonamide, specifically inhibited growth, even in the presence of sulphate. The sulphur starvation symptoms observed with volatile sulphur-containing compounds such as ethanethiol, ethanedithiol, propanethiol and dimethyl sulphide may have been due to the evaporation of these compounds from the medium.

Two groups of enzymes can be distinguished according to their response to sulphur starvation. Cysteine synthase, L-cysteine desulphydrase and thiosulphate reductase always showed an increase in specific activity, whereas the activity of the sulphate activation sequence decreased under severe sulphur stress. This permits differentiation between sulphur starvation as depletion of a usable substrate, and limited growth due to slow metabolic conversion: the activity of the sulphate activation sequence is high under limited growth conditions, but declines under sulphur starvation conditions.

Regulation of the sulphate activation sequence. The sulphate activation sequence in higher plants, green algae and some cyanobacteria consists of ATP-sulphurylase and APS-sulphotransferase (Schmidt, 1979). However, for another group of cyanobacteria and for the eubacteria this sequence involves ATP-sulphurylase, APS-kinase and 3'-phosphoadenosine-5'-phosphosulphate- (PAPS)-sulphotransferase. Increased activity of this enzyme sequence in *C. fusca* was observed with cysteine esters, but not with L- or D-cysteine itself. This was surprising because in *Rhodospseudomonas globiformis* L- or D-cysteine (0.8 mM) increased the activity of the sequence (Imhoff *et al.*, 1981). The *in vivo* activity ATP-sulphurylase of *Anacystis nidulans* was not affected by L-cysteine (10 mM; Sawhney & Nicholas, 1976), and nor was that of *Euglena gracilis* (Brunold & Schiff, 1976). L-Cysteine stimulated the ATP-sulphurylase of *Lemna minor* (0.5 mM; Brunold & Schmidt, 1978), *Ipomoea* (Zink, 1984), and pea and tomato shoots (10 mM; Ellis, 1969), whereas the activity of the enzyme in tobacco cells was decreased after 2 d in the presence of L-cysteine (0.1 mM; Jenni *et al.*, 1980; Trinity *et al.*, 1980). The activity of the APS-sulphotransferase of *Phaseolus vulgaris* decreased after 2 d in the presence of L-cysteine (1 mM; Wyss & Brunold, 1979), and a decrease also occurred in *Lemna minor* after 1 d in the presence of 0.5 mM-L-cysteine (Brunold & Schmidt, 1978) or D-cysteine (Brunold & Suter, 1984).

Increased activity of the sulphate activation sequence during growth with L-cystine was observed for *Lemna gibba*, tomato shoots and roots, turnip shoots and lettuce plants (0.4 mM; Ellis, 1969). In this work, an increase in activity of the sulphate activation sequence was observed for *C. fusca* with sulphate esters and thioethers such as L- and D-methionine, L-methionine sulfoxide and L-methioninol. Such an increase by L-methionine was described only for *Ipomoea* (1 mM; Zink, 1984), *Synechococcus* 6301 (0.2 mM; Krauss, 1984), and pea and tomato shoots (10 mM; Ellis, 1969). In this work we also found increased activity of this sequence during growth on isethionic acid formamidinesulphinic acid and cysteinesulphinic acid, whereas growth on N-acetyl-L-cysteine and cystathionine decreased this activity.

Regulation of cysteine synthase. O-Acetyl-L-serine sulphydrolase and O-acetyl-L-serine-acetate lyase (sulphide adding) are alternative names for cysteine synthase. L-Cysteine, the end product of this enzyme activity, seems to have no influence *in vivo* on the enzyme level of *C. fusca* (0.3 mM; this work), *Synechococcus* 6301 (0.2 mM; Krauss, 1984), *Lemna minor* (0.5 mM; Brunold & Schmidt, 1978), tobacco cells (0.1 mM; Brunold *et al.*, 1981), and *Phaseolus vulgaris* (1 mM; Wyss & Brunold, 1979). *In vitro* inhibition of cysteine synthase with L-cysteine has been measured for *C. fusca* (K_i 0.048 mM; Krauss, 1984), *Triticum* (K_i 2.27 mM; Ascano & Nicholas, 1977) and *Trifolium* (K_i 0.5 mM; Ng & Anderson, 1978); therefore Smith (1975) postulated a regulation of cysteine synthase by the intracellular cysteine pool.

Growth of *C. fusca* on O-acetyl-L-serine in the presence of sulphate, L-cysteine, D-cysteine, D-cystine or S-methyl-L-cysteine for 1 or 4 d did not change the cysteine synthase activity (Krauss, 1984). This finding is in agreement with results from higher plants. For tobacco it was shown that O-acetyl-L-serine increased cysteine synthase activity only in conditions of nitrogen starvation, indicating that this compound is recognized as a nitrogen source (Smith, 1980). In contrast, O-acetyl-L-serine stimulates the cysteine synthase of *Escherichia coli* (1 mM; Jones-Mortimer *et al.*, 1968) and *Salmonella typhimurium* (2 mM; Hulanicka *et al.*, 1979; 0.5 mM; Baptist & Kredich, 1977), and this compound seems to be the effector of the cysteine synthase gene, whereas L-cysteine and sulphide are repressors for this activity in *E. coli* (Jagura-Burdzy & Kredich, 1983) and *S. typhimurium* (Hulanicka *et al.*, 1979).

In spinach, alkylthiols can react with cysteine synthase to form the corresponding cysteine

derivatives (Giovannelli & Mudd, 1968). These could interfere with cysteine-dependent reactions (competitive reactions?) leading to a physiological signal of sulphur shortage; thus sulphur limitation and sulphur starvation have the same expression of high cysteine synthase activity. With *C. fusca*, we observed such an influence with mercaptides such as alkylthiols, DTE, reduced glutathione and the thioethers *S*-methyl-L-cysteine and *S*-benzyl-L-cysteine. The same influence of reduced glutathione was noticed for phototrophic bacteria *in vitro*, but not *in vivo* (1 mM; Hensel, 1979). The increase of this enzyme activity in *C. fusca* by L- or D-homocysteine and L-homocystine is in contrast to phototrophic bacteria, where the enzyme was unchanged by these compounds (10 mM; Hensel, 1979).

A decrease in cysteine synthase activity was observed for our *C. fusca* strain only for growth on methionine sulfoxide (but not on L-methionine), which had no effect on the enzyme of *Lemna paucicostata* (2 μ M; Thompson *et al.*, 1982) and phototrophic bacteria (5 mM; Hensel, 1979).

Oxidized sulphur compounds had no influence on the *C. fusca* cysteine synthase except for formamidinesulphinic acid and sulphanilic acid. *S*-Sulfo-L-cysteine lowered this enzyme activity in phototrophic bacteria (3 mM; Hensel, 1979).

Regulation of L- and D-cysteine desulphydrase. In our experiments, L-cysteine desulphydrase activity showed a regulation pattern similar to that of cysteine synthase. This can be explained by our observation that cysteine synthase also catalyses other reactions, e.g. an isotopic exchange reaction (Schmidt, 1977) and liberation of free sulphide (Krauss, 1984), indicating that L-cysteine desulphydrase is identical with cysteine synthase. An L-cysteine desulphydrase distinct from cysteine synthase was not detected in *C. fusca*. Moreover, L-cysteine could be transformed to β -mercaptopyruvate by transamination or oxidative deamination reactions, yielding the substrate for β -mercaptopyruvate sulphotransferase, whose activity leads to production of sulphide and pyruvate (Schmidt, 1984) thus eliminating the need for an L-cysteine desulphydrase.

The regulation and function of D-cysteine desulphydrase is unknown. This enzyme activity has been described so far only for spinach and *C. fusca* (Schmidt & Erdle, 1983). The enzyme activity in *C. fusca* was not influenced by normal sulphur sources and therefore seems to be constitutive. However, an increase during growth on L-homocystine, L-cysteic acid, sulphanilic acid and *S*-sulphosalicylic acid was observed.

Regulation of thiosulphate reductase. The thiosulphate reductase isolated from *C. fusca* (Schmidt *et al.*, 1984), with so far unknown function, has rhodanese activity. L-Cysteine had no influence on this enzyme in *C. fusca*. D-Cysteine increased its activity in *C. fusca* (Table 2) and *Lemna minor* (0.6 mM; Krauss, 1984). Higher activities were also found using cysteine esters, L-homocystine, cysteamine, cystathionine, *S*-methyl-L-cysteine, dimethyl sulfoxide, sulphate esters and sulphonc acids as sulphur sources. In general, thiosulphate reductase activity seems to be higher under sulphur limitation and thus has regulation behaviour analogous to that of cysteine synthase.

General conclusions. One general principle for control of assimilatory sulphate reduction is a response to sulphur limitation/starvation conditions. Thus enhanced enzyme activities for the sulphate activation sequence, cysteine synthase and thiosulphate reductase can be explained as a consequence of limited availability of sulphur for growth, whereas sulphur starvation can be recognized by a decrease in the activity of the sulphate activation sequence. If a sulphur compound analysed for growth is metabolized slowly, these typical sulphur deficiency symptoms can be expected.

Another mechanism seems to be operative if cysteine analogues prevent utilization of cysteine without affecting sulphate uptake. Such conditions should lead to an increased signal of metabolic activities governed by L-cysteine, such as L-cysteine synthase itself or thiosulphate reductase; thioethers of cysteine seem to inhibit according to such principles.

A third factor is probably the reduction state of the sulphur compound used for growth. If *C. fusca* strain 211-8b is fed with reduced sulphur compounds, their oxidation to sulphate is slow,

leading in some cases to the accumulation of zero-valency sulphur (Krauss *et al.*, 1984). This could lead to sulphate deficiency signals even though reduced sulphur is available, e.g. for L-cyst(e)ine esters or elemental sulphur.

Control of the uptake of sulphur compounds could also regulate the availability of these compounds for growth; however, methods are not available to perform such measurements with non-labelled sulphur compounds. Therefore the interpretation of the data presented in this paper concerning the regulation of enzyme activities could not be focussed on one specific signal.

Since nitrate and sulphate reduction have to be balanced (Reuveny & Filner, 1977), the availability of nitrogen compounds could influence the sulphate reduction sequence and vice versa, enhancing possible signals for the regulation of the sulphate reduction pathway.

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REFERENCES

- ARNON, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidases in *Beta vulgaris*. *Plant Physiology* **24**, 1–15.
- ASCANO, A. & NICHOLAS, D. J. D. (1977). Purification and properties of *O*-acetyl-L-serine sulphydrylase from wheat leaves. *Phytochemistry* **16**, 889–899.
- BAPTIST, E. W. & KREDICH, N. M. (1977). Regulation of L-cystine transport in *Salmonella typhimurium*. *Journal of Bacteriology* **131**, 111–118.
- BRUNOLD, C. & ERISMANN, K. H. (1975). H₂S as sulphur source in *Lemna minor* L. II. Direct incorporation into cysteine and inhibition of sulfate assimilation. *Experientia* **15**, 508–510.
- BRUNOLD, C. & SCHIFF, J. A. (1976). Studies of sulfate utilization by algae. 15. Enzymes of assimilatory sulfate reduction in *Euglena* and their cellular localization. *Plant Physiology* **57**, 430–436.
- BRUNOLD, C. & SCHMIDT, A. (1978). Regulation of sulfate assimilation in plants. 7. Cysteine inactivation of adenosine-5'-phosphosulfate sulfotransferase in *Lemna minor* L. *Plant Physiology* **61**, 342–347.
- BRUNOLD, C. & SUTER, M. (1984). Regulation of sulfate assimilation by nitrogen nutrition in the duckweed *Lemna minor* L. *Plant Physiology* **76**, 579–583.
- BRUNOLD, C., ZYRD, J.-P. & LAVACHY, P. (1981). Regulation of enzymes of assimilatory sulfate reduction in aerated cell suspension cultures of *Nicotiana sylvestris*. *Plant Science Letters* **21**, 167–174.
- ELLIS, R. J. (1969). Sulphate activation by higher plants. *Planta* **88**, 34–42.
- GAITONDE, M. K. (1967). A spectrophotometric method for the direct determination of cysteine in the presence of other naturally occurring amino acids. *Biochemical Journal* **104**, 627–633.
- GIOVANELLI, J. & MUDD, S. H. (1968). Sulfuration of *O*-acetyl-homoserine by two fractions from spinach. *Biochemical and Biophysical Research Communications* **31**, 275–280.
- HART, J. W. & FILNER, P. (1969). Regulation of sulfate uptake by amino acids in cultured tobacco cells. *Plant Physiology* **44**, 1254–1259.
- HENSEL, G. (1979). *O*-Acetylserinsulphydrylase und *S*-sulfocysteinesynthase aus *Chromatium vinosum* und *Rhodospirillum tenue*: molekulare, kinetische und regulatorische Eigenschaften. Thesis, Bonn.
- HODSON, R. C., SCHIFF, J. A. & SCARSELLA, A. J. (1968). Studies of sulfate utilization by algae. 7. *In vivo* metabolism of thiosulfate by *Chlorella*. *Plant Physiology* **43**, 570–577.
- HODSON, R. C., SCHIFF, J. A. & MATHER, J. P. (1971). Studies of sulfate utilization by algae. 10. Nutritional and enzymatic characterization of *Chlorella* mutants impaired for sulfate assimilation. *Plant Physiology* **47**, 306–311.
- HULANICKA, M. D., HALLQUIST, S. G., KREDICH, N. M. & MOJICA, A. T. (1979). Regulation of *O*-acetylserine sulphydrylase B by L-cysteine in *Salmonella typhimurium*. *Journal of Bacteriology* **140**, 141–146.
- IMHOFF, J. F., THEN, J., HASHWA, F. & TRÜPER, H. G. (1981). Sulfate assimilation in *Rhodopseudomonas globiformis*. *Archives of Microbiology* **130**, 234–237.
- JAGURA-BURDZY, G. & KREDICH, N. M. (1983). Cloning and physical mapping of the *cysB* region of *Salmonella typhimurium*. *Journal of Bacteriology* **155**, 578–585.
- JENNI, B. E., BRUNOLD, C., ZYRD, J.-P. & LAVACHY, P. (1980). Properties and regulation of adenosine-5'-phosphosulfate sulfotransferase from suspension cultures of *Nicotiana sylvestris*. *Planta* **150**, 140–143.
- JONES-MORTIMER, M. C., WHELDRAKE, J. F. & PASTERNAK, C. P. (1968). The control of sulphate reduction in *Escherichia coli* by *O*-acetyl-L-serine. *Biochemical Journal* **107**, 51–53.
- KELLOG, W. W., CADLE, R. D., ALLEN, E. R., LAZARUS, A. L. & MARTELL, E. A. (1972). The sulfur cycle. *Science* **175**, 587–596.
- KRAMER, E. & SCHMIDT, A. (1984). Oxidation of cysteine to cystine by membrane fractions of *Chlorella fusca*. *Planta* **160**, 235–241.
- KRAUSS, F. (1984). *Funktion, Eigenschaften und Regulation der multifunktionalen Cysteinsynthase und einiger am Cysteinstoffwechsel beteiligter Enzyme beim Wachstum von Chlorella fusca auf verschiedenen schwefelhaltigen Verbindungen*. Thesis, München.

- KRAUSS, F., SCHÄFER, W. & SCHMIDT, A. (1984). Formation of elemental sulfur by *Chlorella fusca* during growth on L-cysteine ethylester. *Plant Physiology* **74**, 176–182.
- NG, B. H. & ANDERSON, J. W. (1978). Chloroplast cysteine synthase of *Trifolium repens* and *Pisum sativum*. *Phytochemistry* **17**, 879–885.
- RENNENBERG, H. (1981). Differences in the use of cysteine and glutathione as sulfur source in photoheterotrophic tobacco suspension cultures. *Zeitschrift für Pflanzenphysiologie* **105**, 31–40.
- RENNENBERG, H. & UTHEMANN, R. (1980). Effects of L-methionine-S-sulfoximine on growth and glutathione synthesis in tobacco suspension cultures. *Zeitschrift für Naturforschung* **35c**, 945–951.
- REUVENY, Z. & FILNER, P. (1977). Regulation of adenosine triphosphate sulfurylase in cultured tobacco cells. *Journal of Biological Chemistry* **252**, 1858–1864.
- SAITO, S. (1976). Sulfur requirement of *Gonium* (Volvocaceae). *Plant and Cell Physiology* **17**, 407–410.
- SAWHNEY, S. K. & NICHOLAS, J. D. J. (1976). Activation of sulphate in *Anabaena cylindrica*. *Planta* **132**, 189–195.
- SCHMIDT, A. (1972). On the mechanism of photosynthetic sulfate reduction. An APS-sulfotransferase from *Chlorella*. *Archives of Microbiology* **84**, 77–86.
- SCHMIDT, A. (1977). Exchange of cysteine-bound sulfide with free sulfide and cysteine synthase activity in *Chlorella pyrenoidosa*. *Zeitschrift für Pflanzenphysiologie* **84**, 435–446.
- SCHMIDT, A. (1979). Photosynthetic assimilation of sulfur compounds. In *Encyclopedia of Plant Physiology New Series*, Vol. 6, *Photosynthesis II*, pp. 481–496. Edited by M. Gibbs & E. Latzko. Berlin: Springer.
- SCHMIDT, A. (1984). Occurrence of mercaptopyruvate sulfotransferase activity in photosynthetic organisms. *Zeitschrift für Naturforschung* **39c**, 916–921.
- SCHMIDT, A. & ERDLE, I. (1983). A cysteine desulfhydrase specific for D-cysteine from the green alga *Chlorella fusca*. *Zeitschrift für Naturforschung* **38c**, 428–435.
- SCHMIDT, A., ERDLE, I. & KÖST, H.-P. (1982). Changes in C-phycoerythrin in *Synechococcus* 6301 in relation to growth on various sulfur compounds. *Zeitschrift für Naturforschung* **37c**, 870–876.
- SCHMIDT, A., ERDLE, I. & GAMON, B. (1984). Isolation and characterization of thiosulfate reductases from the green alga *Chlorella fusca*. *Planta* **162**, 243–249.
- SIEGEL, L. M. (1965). A direct microdetermination for sulfide. *Analytical Biochemistry* **11**, 126–132.
- SMITH, I. K. (1975). Sulfate transport in cultured tobacco cells. *Plant Physiology* **55**, 303–307.
- SMITH, I. K. (1980). Regulation of sulfate assimilation in tobacco cells. *Plant Physiology* **66**, 877–883.
- THOMPSON, G. A., DATKO, A. H., MUDD, S. H. & GIOVANELLI, J. (1982). Methionine biosynthesis in *Lemna*. *Plant Physiology* **69**, 1077–1083.
- TRINITY, P., VON ARB, C., RHODES, D., YAMAYA, T., SCHMIDT, A. & FILNER, P. (1980). Regulation of APS sulfotransferase and other enzymes of S and N metabolism in cultured tobacco cells. *Plant Physiology* **65**, S-76.
- WYSS, H.-R. & BRUNOLD, C. (1979). Regulation of adenosine-5'-phosphosulfate activity by H₂S and cyst(e)ine in primary leaves of *Phaseolus vulgaris* L. *Planta* **147**, 37–42.
- ZEHNDER, A. J. B. & ZINDER, S. H. (1980). *The Natural Environment and the Biogeochemical Cycles*. pp. 105–145. Berlin: Springer.
- ZINK, M. W. (1984). Regulation of ATP-sulfurylase by various nitrogen and sulfur sources in cultured *Ipomoea* spec. *Canadian Journal of Botany* **62**, 2107–2113.