Utilization of Tetrathionate and ^{35}S -labelled Thiosulphate by Washed Cells of *Chlorobium vibrioforme* f. sp. *thiosulfatophilum*

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The utilization of $\text{S}_4\text{O}_6^{2-}$ by washed cells of *Chlorobium vibrioforme* f. sp. *thiosulfatophilum* was light-dependent. The stoichiometry for $\text{S}_4\text{O}_6^{2-}$ oxidation and $^{14}\text{CO}_2$ assimilation was 1:3.5. Both processes were inhibited by O_2 , but this effect was partially reversed by flushing the reaction mixture with argon. A variety of inhibitors of thiol groups and electron transport, as well as uncouplers and ionophores, restricted these activities. During the oxidation of ^{35}S -labelled $\text{S}_2\text{O}_3^{2-}$ by washed cells, the sulphone S atom (from $\text{S}^{35}\text{SO}_3^{2-}$) was rapidly oxidized to SO_4^{2-} via SO_3^{2-} , whereas the first identifiable product of the oxidation of the sulphane S atom (from $^{35}\text{SSO}_3^{2-}$) was S^{2-} and then elemental sulphur, which was extruded into the medium and then reutilized and oxidized to SO_4^{2-} . A number of inhibitors of thiol groups and respiratory carriers, as well as ionophores, also restricted the oxidation of $\text{S}_2\text{O}_3^{2-}$.

INTRODUCTION

Photosynthetic sulphur bacteria assimilate CO_2 under anaerobic conditions in the light during the oxidation of sulphide and thiosulphate (van Niel, 1931). The green sulphur bacterium *Chlorobium thiosulfatophilum* also uses tetrathionate as an electron donor for CO_2 assimilation (Larsen, 1952, 1953).

Tetrathionate is oxidized to sulphate by many thiobacilli (Roy & Trudinger, 1970) and under anaerobic conditions is metabolized by washed cells of *Thiobacillus neapolitanus* to produce thiosulphate, sulphate and small amounts of trithionate and pentathionate (Trudinger, 1964*a*). The oxidation of tetrathionate by *T. neapolitanus* is very sensitive to thiol-binding reagents (Trudinger, 1965) and is inhibited by oxygen (Trudinger, 1964*b, c*). However, little is known about the mechanism of tetrathionate oxidation by photosynthetic bacteria. Thiosulphate oxidation has been studied extensively in the thiobacilli; under aerobic conditions in the dark, the products are polythionates, elemental sulphur and sulphate (Starkey, 1935; Peck, 1960; Pankhurst, 1964). In the purple sulphur bacteria *Chromatium vinosum* strain D, *Chromatium minus* strain 124 and *Thiocapsa roseopersicina* there is a discriminative oxidation of the sulphone (inner) and sulphane (outer) S atoms of thiosulphate (Smith, 1965; Smith & Lascelles, 1966; Trüper & Pfennig, 1966).

In the present study with washed cells of *Chlorobium vibrioforme* f. sp. *thiosulfatophilum*, we report on the light-dependent oxidation of tetrathionate and CO_2 fixation, as well as the effects of various inhibitors on these processes. The utilization of thiosulphate differentially labelled with ^{35}S in the sulphone or sulphane S atoms is also considered.

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DBP, 2,4-dibromophenol; DCCD, *N,N'*-dicyclohexylcarbodiimide; DIECA, sodium diethyldithiocarbamate; GSH, reduced glutathione; HOQNO, 2-heptyl-4-hydroxyquinoline-*N*-oxide; NDB, chloride, 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole; NEM, *N*-ethylmaleimide; pCMB, *p*-chloromercuribenzoate; PCP, pentachlorophenol; TPB, tetraphenylboron.

METHODS

Bacteria and growth conditions. *Chlorobium vibrioforme* f. sp. *thiosulfatophilum* strain NCIB 8346 (=DSM 264) was cultured in 10 l Pyrex bottles, as described previously (Larsen, 1953). Solutions of 0.01% (w/v) $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$; 0.1% (w/v) $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ and 0.2% (w/v) NaHCO_3 were sterilized separately by passing through sterile Millipore filters (GSWP 02500) and were added to the rest of the heat-sterilized culture medium. The pH was adjusted to 6.8 with a sterile solution of 5% (v/v) H_3PO_4 . Cultures were grown anaerobically by filling the bottles with sterile media, and illuminated with five 100 W incandescent lamps ($1000 \mu\text{E m}^{-2} \text{s}^{-1}$). The cells were harvested at 27000 g at 4 °C at a flow rate of 10 l h^{-1} in a Sorvall Superspeed RC-2 centrifuge fitted with a continuous flow rotor (Ivan Sorvall Inc., Norwalk, Conn., U.S.A.). The cells were washed with 50 mM-sodium phosphate buffer (pH 7.0), recentrifuged and resuspended in the buffer. The washed cells were stored in the dark under argon at 4 °C for 24 h.

$^{14}\text{CO}_2$ incorporation by washed cells. The reaction mixture (2 ml), in 6.5 ml glass vials fitted with Suba-seals, contained sodium phosphate (90 μmol) buffer (pH 7.0), washed cells (0.7 to 0.8 mg protein), NaHCO_3 (7.5 μmol) and $\text{Na}_2\text{S}_4\text{O}_6$ (2 μmol). The vials were then made anaerobic by sparging with argon for 10 min via hypodermic needles inserted into the Suba-seals. After preincubation for 10 min in the dark, 50 $\mu\text{Ci NaH}^{14}\text{CO}_3$ (in 10 μl) was added via a gas-tight syringe and the vials were shaken in the illuminated water bath at 30 °C (approx. $800 \mu\text{E m}^{-2} \text{s}^{-1}$). Samples (100 μl) withdrawn with a gas-tight syringe were filtered through Millipore filters (0.22 μm pore size) and washed with 20 ml 50 mM-sodium phosphate buffer (pH 7.0). The filters were placed in 20 ml scintillation vials and dried at 70 to 80 °C. After cooling, 5 ml scintillation fluid that contained, in toluene, 2,5-diphenyloxazole (3 g l^{-1}) and 1,4-bis-2(4-methyl-5-phenyloxazolyl)benzene (0.3 g l^{-1}) was added to each vial and then radioassayed in a Packard TRI CARB 3375 scintillation spectrometer. Similar experiments were also conducted under aerobic conditions in open vials.

Determination of tetrathionate. $\text{S}_4\text{O}_6^{2-}$ was determined by a modification of the method of Koh (1965) [cited in Roy & Trudinger (1970)]. To samples (100 μl) containing $\text{S}_4\text{O}_6^{2-}$ were added 0.2 M-sodium phosphate buffer (0.4 ml; pH 7.4) and 0.05 M-KCN (0.26 ml); the final pH of the mixture was adjusted to 8.2 with 1 M-KOH. The mixture was incubated at 40 °C for 30 min and then 1.5 M-ferric nitrate in 4 M- HClO_4 (0.3 ml) was added. The mixture was diluted to 2.5 ml with distilled water and the absorbance read at 460 nm in a glass cuvette (1 cm light path) in a Shimadzu QV-50 spectrophotometer, against a blank containing all reagents other than $\text{S}_4\text{O}_6^{2-}$.

Radioassay of ^{35}S compounds in the bacterium. Reaction vials (6.5 ml) fitted with Suba-seals containing Tris/HCl buffer (pH 8.5) (90 μmol), washed cells (0.8 mg protein), NaHCO_3 (15 μmol) and $\text{Na}_2\text{S}_2\text{O}_3$ (5 μmol) were gassed with argon for 10 min, as described above, before incubating in a reciprocating water bath at 30 °C. The vials were uniformly illuminated with 100 W incandescent lamps ($800 \mu\text{E m}^{-2} \text{s}^{-1}$). After a 10 min preincubation, the reaction was initiated by adding 100 $\mu\text{Ci S}_2\text{O}_3^{2-}$ labelled in either the sulphone or the sulphane S atom. Samples (100 μl) withdrawn with a gas-tight microsyringe were filtered through Millipore GSWP 02500 filters, which were then washed with 20 ml Tris/HCl buffer (pH 8.5). Other samples (100 μl) of the reaction mixture were centrifuged at 27000 g for 10 min to remove the bacteria and $^{35}\text{SO}_4$ was determined in the supernatant by adding distilled water (9 ml) and a solution (1 ml) that contained barium chloride (15 g) and 4.8 ml of conc. HCl in 100 ml distilled water. Samples (0.5 ml) of the suspension containing barium sulphate were filtered (Millipore filters, GSWP 02500) and the filters were washed with 50 mM-Tris/HCl buffer (pH 8.5). The filters were placed in 20 ml scintillation vials and dried at 70 to 80 °C. After cooling, radioactivity was determined as described above.

Separation of products of ^{35}S -labelled thiosulphate. The reaction mixture, in 6.5 ml glass vials fitted with Suba-seals, contained 90 μmol Tris/HCl buffer (pH 8.5), washed cells (0.8 mg protein), 0.5 $\mu\text{Ci S}^{35}\text{SO}_3^{2-}$ or $^{35}\text{SSO}_3^{2-}$, 5 $\mu\text{mol Na}_2\text{S}_2\text{O}_3$ carrier and 15 $\mu\text{mol NaHCO}_3$, in a total volume of 2 ml. The vials were gassed with argon and shaken in an illuminated water bath ($800 \mu\text{E m}^{-2} \text{s}^{-1}$) at 30 °C for 10 min and 60 min. The reaction was terminated with 100 μl 0.5 M-N-ethylmaleimide (NEM) and the reaction mixture centrifuged at 10000 g for 15 min at 4 °C. The pellet was suspended in 1 ml 50 mM-Tris/HCl buffer (pH 8.5) and sonicated with an MSE titanium ultrasonic probe at maximum output with five 1 min bursts over a period of 15 min at 4 °C. The sonicate was centrifuged at 27000 g for 15 min at 4 °C and samples of the supernatant (50 μl) were separated by high-voltage paper electrophoresis (1 h, 30 V cm^{-1}) (Tate, 1968). After electrophoresis, the Whatman 3MM paper was dried in hot air and radioactivity located in a Packard 7201 radiochromatogram scanner.

Protein determination. Cell protein was determined by a biuret method (La Rivière, 1958) as modified by Schmidt *et al.* (1963).

Biochemicals. Antimycin A, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), 2-heptyl-4-hydroxyquinoline-*N*-oxide (HOQNO), oligomycin, tetraphenylboron (TPB), rotenone, valinomycin, *N*-ethylmaleimide and *p*-chloromercuribenzoate (pCMB) were purchased from Sigma, pentachlorophenol (PCP) from Merck, *N,N'*-dicyclohexylcarbodiimide (DCCD) and sodium tetrathionate from Koch-Light, 2,4-dibromophenol (DBP) from ICN Pharmaceuticals (Cleveland, Ohio, U.S.A.), 2,4-dinitrophenol from Ajax (Sydney, Australia), and 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD chloride) from Aldrich (Milwaukee, Wis., U.S.A.).

Radiochemicals. Carrier-free $\text{NaH}^{14}\text{CO}_3$ ($55.7 \text{ mCi mmol}^{-1}$; $2.06 \text{ GBq mmol}^{-1}$), $\text{Na}_2^{35}\text{SSO}_3$ (38 mCi mmol^{-1} ; $1.41 \text{ GBq mmol}^{-1}$) and $\text{Na}_2\text{S}^{35}\text{SO}_3$ (25 mCi mmol^{-1} ; $0.92 \text{ GBq mmol}^{-1}$) were obtained from Amersham.

RESULTS

Utilization of tetrathionate

The oxidation of $\text{S}_4\text{O}_6^{2-}$ by washed cells exposed to light under anaerobic conditions was proportional to the biomass up to 10 mg protein . Maximum utilization was observed at pH 7.0 in $50 \text{ mM-phosphate buffer}$, while in Tris/HCl buffer of identical molarity utilization decreased markedly over the pH range 7.0 to 8.5. The oxidation showed typical saturation kinetics, with a K_m value of 0.32 mM for $\text{S}_4\text{O}_6^{2-}$ estimated from double reciprocal plots. The maximum rates of oxidation of $\text{S}_4\text{O}_6^{2-}$ and of incorporation of $^{14}\text{CO}_2$ were 12 and 42 nmol min^{-1} , respectively, with a stoichiometry of $1:3.5$ at the end of the reaction (Fig. 1).

The oxidation of $\text{S}_4\text{O}_6^{2-}$ under anaerobic conditions was light-dependent (Fig. 2). $\text{S}_4\text{O}_6^{2-}$ was not utilized by cells in the dark, but on exposure to light it was readily oxidized. At a low light intensity ($<250 \mu\text{E m}^{-2} \text{ s}^{-1}$) the rates of $\text{S}_4\text{O}_6^{2-}$ oxidation and $^{14}\text{CO}_2$ assimilation were only one-tenth of the optimum rates recorded at $800 \mu\text{E m}^{-2} \text{ s}^{-1}$.

$\text{S}_4\text{O}_6^{2-}$ oxidation and $^{14}\text{CO}_2$ assimilation by washed cells were inhibited by O_2 (Fig. 3). A decrease in the concentration of O_2 resulted in a corresponding increase in $^{14}\text{CO}_2$ fixation and oxidation of $\text{S}_4\text{O}_6^{2-}$; maximum rates were observed under anaerobic conditions. The addition of 1% (v/v) O_2 in argon to washed cells after 60 min incubation under argon diminished the oxidation of $\text{S}_4\text{O}_6^{2-}$ and the assimilation of $^{14}\text{CO}_2$ by 50% . When washed cells were incubated with 1% (v/v) O_2 in argon from the outset, the rates for the two processes were diminished by 40% .

The effects of various inhibitors on the oxidation of $\text{S}_4\text{O}_6^{2-}$ and the assimilation of $^{14}\text{CO}_2$ by washed cells are presented in Table 1. NEM (1 mM), pCMB (1 mM) and iodoacetamide (5 mM) markedly inhibited the two processes. The inhibition by these thiol-binding compounds was not reversed by reduced glutathione (GSH). The uncoupling agents CCCP and DNP, together with DCCD and TPB, inhibited both the oxidation of $\text{S}_4\text{O}_6^{2-}$ and the assimilation of $^{14}\text{CO}_2$ at low concentrations (Table 1). A number of inhibitors of electron transport were also effective against these processes (Table 2). Rotenone (0.25 mM) restricted $^{14}\text{CO}_2$ assimilation to a greater extent than $\text{S}_4\text{O}_6^{2-}$ uptake, whereas antimycin A ($250 \mu\text{g ml}^{-1}$), HOQNO ($32 \mu\text{M}$), azide (5 mM) and cyanide (0.1 mM ; results not shown) were equally effective in inhibiting both processes. Partial inhibitions of oxidation and assimilation were also exhibited by amytal (1.5 mM) or atebirin ($250 \mu\text{M}$). In addition, oligomycin ($100 \mu\text{g ml}^{-1}$) and NBD chloride ($25 \mu\text{g ml}^{-1}$) restricted both processes. The ionophores valinomycin, TPB and nigericin inhibited $^{14}\text{CO}_2$ assimilation to a greater extent than $\text{S}_4\text{O}_6^{2-}$ oxidation. The inhibitors thiourea, sodium diethyldithiocarbamate (DIECA) and *o*-phenanthroline all diminished the uptake of $\text{S}_4\text{O}_6^{2-}$ and the assimilation of $^{14}\text{CO}_2$.

Utilization of ^{35}S -labelled thiosulphate

The uptake of $^{35}\text{SSO}_3^{2-}$ and $\text{S}^{35}\text{SO}_3^{2-}$ by washed cells is presented in Fig. 4. The utilization of $^{35}\text{SSO}_3^{2-}$ under anaerobic conditions in the light resulted in a gradual increase in the incorporation of the radioactivity into the cells, and after 1 h elemental sulphur was released into the medium. After a further 60 min , the sulphur was re-utilized at a slower rate and produced SO_4^{2-} . During the oxidation of $\text{S}^{35}\text{SO}_3^{2-}$, ^{35}S was rapidly incorporated into SO_4^{2-} , of which about 90% was found in the reaction medium after 90 min . There were negligible amounts of radioactivity in the bacteria.

Washed cells were incubated for 10 min and 60 min with $^{35}\text{SSO}_3^{2-}$ or $\text{S}^{35}\text{SO}_3^{2-}$ and the cell products were separated by high-voltage paper electrophoresis, as described in Methods. S^{2-} (as the NEM complex) and elemental sulphur were detected in the samples incubated for 10 min with $^{35}\text{SSO}_3^{2-}$ and after 60 min $^{35}\text{SO}_4^{2-}$ was produced. However, with $\text{S}^{35}\text{SO}_3^{2-}$, the main

Fig. 1. Stoichiometry of the oxidation of $\text{S}_4\text{O}_6^{2-}$ and assimilation of CO_2 by washed cells. Washed cells from cultures grown anaerobically in the light as described in Methods were incubated in 6.5 ml vials fitted with Suba-seals. The flasks were gassed with argon and shaken in an illuminated water bath at 30°C ($800 \mu\text{E m}^{-2} \text{s}^{-1}$). The reaction mixture (2 ml) contained washed cells (0.7 mg protein) and $7.5 \mu\text{mol NaHCO}_3$. The reaction was started by adding $2 \mu\text{mol Na}_2\text{S}_4\text{O}_6$ with a Hamilton microsyringe. $\text{S}_4\text{O}_6^{2-}$ was determined in portions ($100 \mu\text{l}$) of the reaction mixture, withdrawn at 15 min intervals over a 200 min period (●). The assimilation of $^{14}\text{CO}_2$ (○) was determined in similar vials over the same time period, except that the reaction was initiated by adding $50 \mu\text{Ci NaH}^{14}\text{CO}_3$ as described in Methods.

Fig. 2

Fig. 3

Fig. 2. Effect of light on $\text{S}_4\text{O}_6^{2-}$ oxidation by washed cells. Washed cells grown anaerobically in the light were incubated in 6.5 ml vials fitted with Suba-seals, gassed with argon and shaken in an illuminated water bath at 30°C ($800 \mu\text{E m}^{-2} \text{s}^{-1}$). The reaction mixture (2 ml) contained washed cells (0.75 mg protein) and $7.5 \mu\text{mol NaHCO}_3$. The reaction was initiated by adding $2 \mu\text{mol Na}_2\text{S}_4\text{O}_6$ with a gas-tight microsyringe. Two vials (●, □) were illuminated from zero time; one of them (□) was darkened after 1 h and reilluminated 1 h later. A third vial (○) was kept in the dark throughout the experiment, while a fourth (■) was maintained in the dark for 90 min and then illuminated. The arrows indicate the turning on and off of the lights.

Fig. 3. Effect of O_2 on $\text{S}_4\text{O}_6^{2-}$ oxidation by washed cells. Washed cells from cultures grown anaerobically in the light were incubated in 6.5 ml vials and placed in an illuminated water bath at 30°C . Washed cells (0.7 mg protein) were added to the reaction mixture (2 ml) containing $15 \mu\text{mol NaHCO}_3$ and $4 \mu\text{mol Na}_2\text{S}_4\text{O}_6$. One vial (●) was fitted with a Suba-seal and sparged with argon; a second vial (○) was open to the air, and a third (■) was initially incubated in air but filled with argon after 90 min. A fourth vial (□) was filled with 1% (v/v) O_2 in argon at the start of the experiment. The gas phase in the remaining vials (▽, ▼) was initially argon but was changed to 1% (▽) or 3% (▼) O_2 in argon after 1 h.

Table 1. *Effects of various inhibitors on the oxidation of $S_4O_6^{2-}$ and assimilation of $^{14}CO_2$ by washed cells*

The reaction mixture used is given in the legend to Fig. 1. The following procedure was used for each inhibitor: 1 to 50 μ l inhibitor was injected into the reaction mixture and after 10 min preincubation, 2 μ mol $S_4O_6^{2-}$ was added to initiate the reaction. In the absence of inhibitor, the values for $S_4O_6^{2-}$ oxidation and $^{14}CO_2$ assimilation were 12 and 42 nmol min⁻¹, respectively.

Inhibitor	Final concn	Inhibition (%)	
		$S_4O_6^{2-}$ oxidation	$^{14}CO_2$ assimilation
pCMB	0.1 mM	0	10
	1.0 mM	87	57
NEM	0.1 mM	20	30
	1.0 mM	75	72
Iodoacetamide	0.1 mM	66	64
	5.0 mM	100	96
DCCD	10 μ M	44	20
	1 mM	75	100
CCCP	25 μ M	40	80
	50 μ M	80	95
DNP	25 μ M	75	50
	125 μ M	100	100
TPB	25 μ M	25	46
	250 μ M	100	100

Table 2. *Effects of inhibitors of electron transport on the oxidation of $S_4O_6^{2-}$ and assimilation of $^{14}CO_2$ by washed cells*

All inhibitors were dissolved in 95% (v/v) ethanol and appropriate controls with equivalent volumes of ethanol were included for each inhibitor. Experimental details were as described for Table 1 and Fig. 1.

Inhibitor	Final concn	Inhibition (%)	
		$S_4O_6^{2-}$ oxidation	$^{14}CO_2$ assimilation
Rotenone	0.05 mM	22	100
	0.25 mM	50	100
HOQNO	0.8 μ M	20	25
	32.0 μ M	45	50
Antimycin A	125 μ g in 2 ml	35	50
	500 μ g in 2 ml	100	100
Amytal	1.5 mM	40	60
Atebrin	25.0 μ M	50	34
	250.0 μ M	53	41
Azide	0.1 mM	25	10
	5.0 mM	75	70

products were SO_3^{2-} (trapped as NEM complex) and SO_4^{2-} after both incubation periods (10 and 60 min).

The thiol-binding compounds iodoacetamide (1.0 mM), pCMB (0.1 mM), NEM (1.0 mM) and sulphite (1.0 mM) all inhibited $S_2O_3^{2-}$ uptake (Table 3). These effects were not, however, reversed by GSH. Electron transport chain inhibitors also restricted the oxidation of $S_2O_3^{2-}$ (Table 3). Thus amytal (7.5 μ M), HOQNO (50 μ M) and antimycin A (5 μ g ml⁻¹) inhibited at relatively low concentrations, whereas rotenone (1 mM), azide (1 mM) and cyanide (0.1 mM) (results not shown) did so at higher concentrations.

The oxidation of $^{35}SSO_3^{2-}$ was enhanced by S^{2-} (Fig. 5); a 100-fold stimulation was observed with 1 mM- S^{2-} .

Fig. 4. Incorporation of sulphane ^{35}S -labelled and sulphane ^{35}S -labelled thiosulphate into washed cells and extrusion of $^{35}\text{SO}_4^{2-}$ into the medium. Washed cells from cultures grown anaerobically in the light were incubated in 6.5 ml glass vials under anaerobic conditions at a light intensity of $800 \mu\text{E m}^{-2} \text{s}^{-1}$. The reaction mixture (2 ml) contained cells (0.8 mg protein), $15 \mu\text{mol NaHCO}_3$ and $5 \mu\text{mol Na}_2\text{S}_2\text{O}_3$. The reaction was initiated by adding $100 \mu\text{Ci}$ (5 mM) $\text{Na}_2\text{S}_2\text{O}_3^{2-}$ in $10 \mu\text{l}$, labelled in the sulphane S atom – $^{35}\text{SSO}_3^{2-}$ (a) or the sulphane S atom – $\text{S}^{35}\text{SO}_3^{2-}$ (b). The incorporation of the tracer into the washed cells ($\circ$) and into SO_4^{2-} extruded by the cells ($\bullet$) were determined as described in Methods.

Table 3. *Effects of thiol-binding compounds and inhibitors of electron transport on $^{35}\text{SSO}_3^{2-}$ oxidation by washed cells*

Experimental details were as described for Fig. 4. The inhibitors rotenone, amytal, HOQNO and antimycin A were dissolved in 95% (v/v) ethanol; appropriate controls with equivalent volumes of ethanol were included for such inhibitors. In the absence of inhibitor the rate of oxidation of $^{35}\text{SSO}_3^{2-}$ was 15 nmol min^{-1} .

Inhibitor	Final concn	Inhibition (%)	Inhibitor	Final concn	Inhibition (%)
Iodoacetamide	0.1 mM	66	Rotenone	0.1 mM	25
	1.0 mM	74		1.0 mM	40
NEM	0.1 mM	50	Amytal	0.7 μM	30
	1.0 mM	85		7.5 μM	40
pCMB	0.1 mM	47	HOQNO	10 μM	38
	1.0 mM	75		50 μM	55
Sulphite	0.1 mM	60	Antimycin A	10 μg in 2 ml	68
	1.0 mM	87		50 μg in 2 ml	75
			Azide	0.1 mM	25
				1.0 mM	75

DISCUSSION

A stoichiometry of 1:3.5 for $\text{S}_4\text{O}_6^{2-}$ oxidation and CO_2 assimilation reported herein indicates that the assimilatory process may be represented by the following equation:

There have been conflicting reports about the operation of the reductive pentose phosphate cycle in *Chlorobium* (Evans *et al.*, 1966; Sirevåg, 1974; Tabita *et al.*, 1974). The view now widely held is that the reductive carboxylic cycle is the main pathway of CO_2 assimilation in this organism (Buchanan & Sirevåg, 1976; Takabe & Akazawa, 1977; Fuchs *et al.*, 1980a, b; Ivanovsky *et al.*, 1980). Takabe & Akazawa (1977) found that CO_2 assimilation was very sensitive to O_2 , perhaps as a result of inhibition by O_2 of the two ferredoxin-dependent enzymes of the reductive carboxylic cycle, namely pyruvate synthase and 2-oxoglutarate synthase. Although we have confirmed the inhibition of CO_2 fixation by O_2 , we find that this

Fig. 5. Effects of various concentrations of S^{2-} on the uptake of $^{35}SSO_3^{2-}$ by washed cells. The reaction mixture was that described in the legend to Fig. 4, except that various concentrations of S^{2-} were added. □, $^{35}SSO_3^{2-}$ alone; ■, $^{35}SSO_3^{2-}$ + 0.2 mM- S^{2-} ; ○, $^{35}SSO_3^{2-}$ + 1 mM- S^{2-} ; ●, $^{35}SSO_3^{2-}$ + 2 mM- S^{2-} .

effect can be reversed by flushing with argon, contrary to reports of an irreversible effect (Takabe & Akazawa, 1977).

At low light intensity ($<250 \mu E m^{-2} s^{-1}$) $S_4O_6^{2-}$ oxidation was very slow, presumably because ATP production would then be inadequate for CO_2 fixation. At higher light intensities, with attendant increases in ATP supply, CO_2 fixation and $S_4O_6^{2-}$ oxidation were found to be maximal. Uncouplers and electron transport inhibitors restricted both these processes to a similar extent, indicating that the oxidation of $S_4O_6^{2-}$ is mandatory for the functioning of the electron transport chain and its attendant phosphorylation.

In the photosynthetic purple sulphur bacteria *Chromatium vinosum* strain D, *Chromatium minus* strain 124 and *Thiocapsa roseopersicina*, the sulphone and sulphane S atoms of $S_2O_3^{2-}$ are oxidized to SO_4^{2-} at different rates (Smith & Lascelles, 1966; Trüper & Pfennig, 1966). An initial reductive cleavage of $S_2O_3^{2-}$ as found in thiobacilli (Peck, 1960, 1962) was also observed in the *Chromatiaceae* (Smith, 1964, 1965; Smith & Lascelles, 1966). In *Chlorobium vibrioforme* f. sp. *thiosulfatophilum* we have also found a discriminative oxidation of the S atoms of $S_2O_3^{2-}$: thus the sulphone S atom was oxidized at a faster rate than the sulphane S atom. The results therefore indicate a reductive cleavage of $S_2O_3^{2-}$ with the initial production of S^{2-} and SO_3^{2-} , which are then oxidized to SO_4^{2-} .

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