

Mechanism of Oxidation of Dimethyl Disulphide by *Thiobacillus thioparus* Strain E6

By NEIL A. SMITH AND DON P. KELLY*

Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, UK

(Received 6 June 1988; revised 7 July 1988)

A recently isolated organism, capable of chemolithoautotrophic growth on dimethyl disulphide, was characterized as a strain of *Thiobacillus thioparus*. It had DNA with a base composition of 60.5 ± 1.0 mol% G + C, and ubiquinone-8 (UQ-8) as its only respiratory quinone. Its growth in chemostat culture (at a growth rate of $0.07\text{--}0.08$ h⁻¹) showed yields of 14.4, 11.8 and 2.45 g cell-carbon per mol of dimethyl disulphide (DMDS), dimethyl sulphide (DMS) and thiosulphate, respectively. This is consistent with energy generation from the oxidation of the methyl and the sulphide moieties of DMDS, with oxidation of sulphide to sulphate contributing a yield of about 2.8 g cell-carbon mol⁻¹. From whole organism and cell-free extract studies, DMDS oxidation was shown to proceed by its (NADH-stimulated) reduction to methanethiol (MT), which was oxidized via sulphide, formaldehyde and formate to CO₂ and sulphate by MT oxidase, formaldehyde and formate dehydrogenases, and an uncharacterized sulphide-oxidizing system. The MT oxidase had a K_m of 9.7 μM and showed substrate inhibition with a K_i of about 8 μM. The essential role of catalase during growth on DMDS was shown by the sensitivity of growth on DMDS (but not on thiosulphate) to 3-amino-1,2,4-triazole. Catalase is believed to destroy the peroxide produced by the MT oxidase reaction. DMDS-grown organisms oxidized sulphide, thiosulphate and tetrathionate (with the latter indicated to be an intermediate in thiosulphate oxidation), suggesting the pathway of sulphide oxidation to be similar to that in some other thiobacilli. Carbon assimilation was by the Calvin cycle, with ribulose biphosphate carboxylase being present in cell-free extracts at a specific activity of 80 nmol CO₂ fixed min⁻¹ (mg protein)⁻¹. Hydroxypyruvate reductase (HPR) was not detected at levels sufficient to indicate any role in primary carbon assimilation.

INTRODUCTION

In a previous paper (Smith & Kelly, 1988) we described the isolation of a strain of *Thiobacillus* that could grow chemolithoautotrophically on DMDS as its sole source of energy. On the basis of its physiological behaviour, we then proposed that DMDS was probably oxidized by a pathway involving MT, formaldehyde, formate and sulphide (but not DMS) as intermediates. By analogy with the metabolism of dimethyl sulphoxide, it seemed likely that the initial step in DMDS degradation could be its reductive cleavage to two molecules of MT, with these subsequently being oxidized by the pathway shown in some hyphomicrobia (De Bont *et al.*, 1981; Suylen *et al.*, 1986, 1987). Essential enzymes of such a pathway in the *Thiobacillus* would be a DMDS reductase, an MT oxidase, formaldehyde and formate dehydrogenases, and a sulphide-oxidizing system. Catalase would also be expected to be present, with the role of destroying H₂O₂ produced by the MT oxidase (Suylen *et al.*, 1986, 1987).

Abbreviations: CTAB, cetyltrimethylammonium bromide; DMDS, dimethyl disulphide; DMS, dimethyl sulphide; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; HPR, hydroxypyruvate reductase; HQNO, 2-heptyl-4-hydroxyquinoline-*N*-oxide; MT, methanethiol; NEM, *N*-ethylmaleimide; TOC, total organic carbon.

We also proposed that growth on DMDS was wholly autotrophic, with CO₂ being fixed by the Calvin cycle, and that there was not likely to be any contribution from the serine pathway that has been shown to be essential for growth of the hyphomicrobia on methylated sulphides (Suylen *et al.*, 1986).

We now present enzymological evidence for these proposals.

METHODS

Media and culture conditions. The mineral salts medium used for all batch and continuous cultures contained (g l⁻¹): K₂HPO₄, 2.0; KH₂PO₄, 2.0; NH₄Cl, 0.4; Na₂CO₃, 0.4; MgCl₂.6H₂O, 0.2; 3 ml vitamin mixture (Kanagawa *et al.*, 1982) and 1 ml of trace metal solution (Tuovinen & Kelly, 1973); initial pH 7.1. Strain E6 (Smith & Kelly, 1988) was maintained either in liquid medium on 2 mM-DMDS or as a chemolithoautotroph on solid medium prepared by adding sodium thiosulphate (20 mM) and agar (1.5%, w/v) to the mineral salts medium.

Batch cultures (50 ml) were grown in 250 ml Quickfit flasks sealed with rubber Suba-seal stoppers. DMDS (2 mM) was injected into cultures using a Hamilton syringe.

Chemostat culture work was done in a 1 litre water-jacketed glass vessel, essentially using the system described previously (Smith & Kelly, 1988). The inoculum culture (20%, v/v) was grown in standard batch culture, but because of the volatility of the substrate, growth of the culture could not be established in the chemostat vessel by a period of batch growth. Instead, the culture was pulsed with DMDS-containing medium for 20 min in every hour until a satisfactory biomass was achieved before establishing continuous culture at a dilution rate of 0.07–0.08 h⁻¹.

Respiration measurements. Oxygen uptake rates were determined as described previously (Smith & Kelly, 1988), using suspensions of organisms removed directly from a DMDS-limited chemostat. In experiments to determine the inhibitory effects of FCCP (1–5 μM), NEM (10–100 μM) and HQNO (5–100 μM) on thiosulphate and tetrathionate oxidation by DMDS-grown organisms, the inhibitors were added either during substrate oxidation or 2–4 min before addition of substrate.

Analytical methods. Dry weights, total organic carbon (TOC) and cellular protein were assayed as described previously (Smith & Kelly, 1988). Thiosulphate was determined cyanolytically and thiocyanate was measured by means of the ferric nitrate reagent (Kelly *et al.*, 1969). DMS and DMDS were assayed as before (Kanagawa & Kelly, 1986; Smith & Kelly, 1988). MT solutions were freshly prepared as described by Suylen *et al.* (1986).

DMDS, DMS and MT were also determined by gas chromatography (GC), using a Pye Unicam series 204 gas chromatograph, linked to a model PU 4810 computing integrator, and fitted with a 1 m teflon column (3 mm internal diameter) packed with acetone-washed Porapak QS. Detection was by means of a flame photometric detector with a sulphur filter allowing light transmission at 394 nm. Injection, column and detector temperatures were 200, 100 and 250 °C, respectively. Gas flow rates (ml min⁻¹) were: carrier nitrogen, 40; air, 50; and hydrogen, 70. For analyses, gaseous samples (100 μl) were injected. Compounds were identified by comparison of retention times of experimental unknowns with those of authentic compounds.

Time-dependent appearance of MT from DMDS was measured by headspace GC analysis during anaerobic incubation of cell suspensions. Organisms (7.2 mg protein), harvested (21 000 g, 15 min) from 200 ml of steady-state chemostat culture (1.9 mM-DMDS; *D* 0.07 h⁻¹), were washed and resuspended in mineral salts medium (10 ml). This was dispensed equally into two 23 ml glass universal bottles, sealed with rubber Suba-seal stoppers. One bottle was deoxygenated by bubbling with nitrogen, then both were allowed to equilibrate at 30 °C. DMDS (10 μmol) was injected into each and the appearance of MT measured as described.

Time-dependent disappearance of MT (5 μmol) during aerobic and anaerobic incubations was assayed in parallel experiments using the same procedures.

Chromosomal DNA was extracted and purified according to Beji *et al.* (1987), and its mol% G + C content determined spectrophotometrically according to the methods of Fredericq *et al.* (1961) and Ulitzur (1972).

The ubiquinone fraction of the organism was prepared as described by Wood & Kelly (1985), using the procedure based on method 3 of DiSpirito *et al.* (1983). The resulting solution of ubiquinone in ethanol was chromatographed on Merck DC Alufolien Kieselgel 60F254 thin-layer plates, developed in benzene for 40–50 min, and the ubiquinone visualized by its quenching of UV light at 254 nm. Standards were ubiquinone Q-10 from Sigma, and isolated from *Thiobacillus ferrooxidans*, and Q-8 isolated from *Thiobacillus denitrificans*, *T. tepidarius* and *T. aquaesulis*.

Preparation of cell-free extracts. About 5 g wet wt of organisms, harvested from DMDS-limited chemostat culture, were suspended in 15 ml mineral salts medium and passed twice through a chilled French pressure cell at 140 MPa. The supernatant solution obtained from centrifuging at 35 000 g for 30 min at 5 °C was used as the crude cell-free extract for enzyme assays.

Enzyme assays. All spectrophotometric assays were done at 30 °C using a Pye Unicam SP 1700 spectrophotometer fitted with a water-circulating cuvette block. Ribulose-1,5-bisphosphate carboxylase was

assayed either by (a) the whole organism permeabilization method (Kelly & Wood, 1982), using 0.1% (w/v) cetyltrimethylammonium bromide (CTAB) rather than Triton X-100 as the permeabilizing agent; or (b) measuring the time course of ribulose-bisphosphate-dependent $^{14}\text{CO}_2$ -fixation by the crude cell-free extract. HPR was assayed according to Kelly & Wood (1984), NAD(P) $^+$ -dependent formate dehydrogenase according to Patel & Hoare (1971), NAD(P) $^+$ -dependent formaldehyde dehydrogenase as described by Stirling & Dalton (1978), and catalase by the method of Haywood & Large (1981). MT oxidase (also known as methyl mercaptan oxidase) was assayed (a) by measuring oxygen consumption essentially as described by Suylen *et al.* (1987); and (b) as the time-dependent disappearance of MT during incubation of aerobic suspensions of organisms in sealed vessels. NADH-dependent DMDS reductase was assayed as the time-dependent appearance of MT during anaerobic incubation of suspensions of CTAB-permeabilized organisms: suspensions (1 ml, 3.6 mg protein) were incubated under nitrogen at 30 °C for 20 min in sealed 23 ml universal bottles following addition of 0.5 ml 0.25% (v/v) CTAB. NADH (20 μmol in 0.5 ml) was then added and the mixture agitated. Reaction was initiated by adding DMDS (2 μmol in 40 μl potassium phosphate buffer, pH 7.0). All solutions were deoxygenated with nitrogen before use. MT release into the headspace was determined during subsequent incubation and activity expressed as $\text{nmol MT produced min}^{-1} (\text{mg protein})^{-1}$, after correction for MT production in the absence of NADH.

Special chemicals. MT and thiocyanate were obtained from Fisons, DMS from Aldrich, and DMDS from Sigma.

RESULTS

Taxonomic identity, DNA base composition and ubiquinone of strain E6. As previously described (Smith & Kelly, 1988), strain E6 is morphologically and physiologically an autotrophic, chemolithotrophic *Thiobacillus*, incapable of growth on organic compounds containing carbon-carbon bonds. It differs from the majority of thiobacilli studied in being able to grow autotrophically on one-carbon sulphur compounds, such as the methylated sulphides. In common with *T. thioparus* (Kelly & Harrison, 1988) it can also grow on thiocyanate, a property not demonstrated in the other aerobic thiobacilli. Analysis of the DNA from strain E6 showed a mean mol% G + C content of 60.5 ± 1.0 (from seven estimations by two procedures). This is in the normal range for *T. thioparus* (Kelly & Harrison, 1988). Thin-layer chromatography of its ubiquinone fraction showed ubiquinone-8 (coenzyme Q-8) to be the only quinone detectable. This is also a characteristic of *T. thioparus*. Consequently, we propose that strain E6 is currently best described as a strain of *Thiobacillus thioparus*.

Evidence for a DMDS reductase. Suspensions of *T. thioparus* strain E6, harvested from DMDS-limited chemostat culture, produced MT from DMDS when incubated under anaerobic conditions (Fig. 1a). There was a significant lag before MT production into the headspace gas commenced, possibly due to equilibration between solution and gas phases. This lag was slightly decreased when formaldehyde was also supplied (as a possible source of reductant), but neither the rate nor extent of MT production was as great in its presence. Adding further DMDS to the formaldehyde-supplemented suspension resulted in immediate rapid production of more MT (Fig. 1a). In the absence of formaldehyde, the rate of MT production in the headspace was $35 \text{ nmol min}^{-1} (\text{mg cell-protein})^{-1}$, and the total MT produced was about 3 μmol . The maximum that might be produced from the added DMDS was 20 μmol . Given the extremely high solubility of MT in water (about 480 mM in water saturated with MT at 20 °C; *Merck Index 1968*), the amount of DMDS reduced to MT was probably significantly greater than the 15% observed.

Suspensions of organisms permeabilized by CTAB showed a low initial rate of MT production from DMDS, but its formation increased progressively with time (Fig. 1b). In the presence of NADH (as a reductant), the rate was linear and was initially about six times faster than that in its absence (Fig. 1b). With NADH, total MT production into the headspace was about 2.2 μmol of a possible 4 μmol . Again, a significant quantity of MT was probably also retained in solution. The rate of MT formation from DMDS was thus in the range 6–10 $\text{nmol min}^{-1} (\text{mg protein})^{-1}$ under these conditions.

Cell-free extracts did not show any DMDS-dependent stimulation of NADH or NADPH oxidation when assayed at 25–45 °C, pH 6.0–8.0 in 50–150 mM-phosphate buffers with 0.2–20 μM -DMDS.

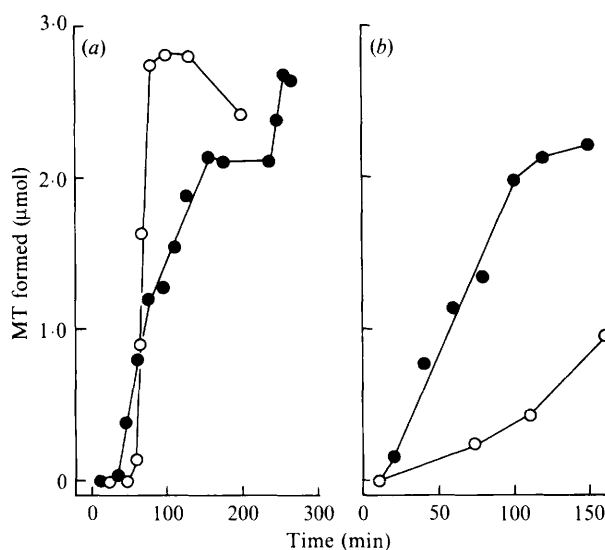


Fig. 1. Formation of MT from DMDS by (a) suspensions of intact organisms and (b) CTAB-treated suspensions of *T. thioparus* strain E6. (a) DMDS (10 μmol) was added to 5 ml of cell suspension (3.5 mg protein ml⁻¹) under a headspace of 18 ml of nitrogen gas. MT in the headspace was measured using suspensions incubated at 30 °C in the absence (○) and presence (●) of 0.5 mM-formaldehyde. A further 10 μmol of DMDS was added to the latter at 240 min. (b) A suspension (5 ml) treated with 0.08% (w/v) CTAB was incubated with DMDS (2 μmol) in the absence (○) and presence (●) of 4 mM-NADH.

Table 1. *Enzymes potentially involved in MT oxidation by T. thioparus strain E6*

Enzyme	Specific activity in crude extract [nmol min ⁻¹ (mg protein) ⁻¹]
MT oxidase (oxygen uptake rate)	67.0
Formaldehyde dehydrogenase	
NAD ⁺	22.4
NADP ⁺	1.0
Formate dehydrogenase	
NAD ⁺	101.3
NADP ⁺	0
NADH oxidase	43.5
NADPH oxidase	43.5
Catalase	3369

Enzymes of MT oxidation. Activities of the key enzymes assayed are shown in Table 1. MT oxidase showed maximum activity at 10–20 μM-MT (and was assayed at 15 μM in Table 1). Substrate inhibition was seen at MT concentrations above 15 μM (Fig. 2a). K_m and V_{max} values were computed from Lineweaver–Burk and Eadie–Hofstee equations as $K_m = 9.7 \pm 1.6$ μM-MT and $V_{max} = 122 \pm 12$ nmol min⁻¹ (mg extract protein)⁻¹, respectively. Assuming the substrate inhibition to be competitive, the K_i for MT (computed for the inhibitory concentrations of MT) was approximately 8 μM.

Both formaldehyde and formate dehydrogenases were NAD-specific and a very high level of catalase was found (Table 1).

Other enzymes of one-carbon metabolism. Ribulose biphosphate carboxylase was present in permeabilized whole cells at a specific activity of 33 nmol CO₂ fixed min⁻¹ (mg dry wt)⁻¹ (Smith & Kelly, 1988). This was equivalent to an activity of 53 nmol min⁻¹ (mg cell-protein)⁻¹. Assaying a crude extract prepared with the French pressure cell gave an activity of 80 nmol min⁻¹ (mg extract-protein)⁻¹.

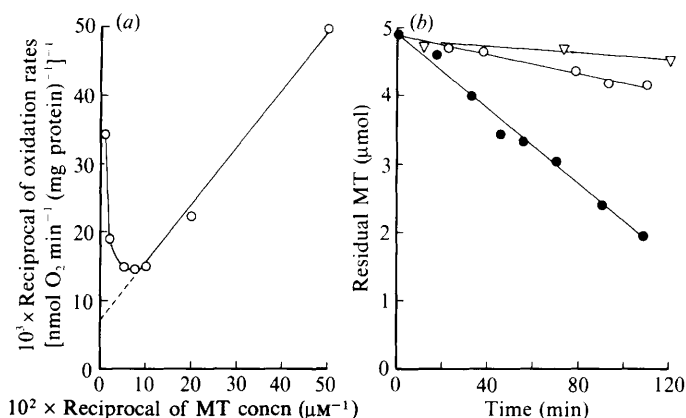


Fig. 2. MT metabolism by extracts and intact suspensions of *T. thioparus* strain E6. (a) MT oxidation by crude cell-free extract. Oxidation rates for concentrations between 2–100 μM are given as a Lineweaver-Burk double reciprocal plot. (b) Consumption of MT (4.9 μmol) by suspensions (5 ml, 3.6 mg protein) incubated under 15 ml of air (●) or nitrogen (○) was measured. An anaerobic control with no organisms (▽) was also tested. Rates of disappearance were 27.8 and 6.8 $\text{nmol min}^{-1} (\text{mg protein})^{-1}$, aerobically and anaerobically, respectively.

HPR activity was detected as 6.5 $\text{nmol NADH oxidized min}^{-1} (\text{mg protein})^{-1}$, against a background of 43.5 for NADH oxidation in the absence of hydroxypyruvate. No NADPH-dependent HPR activity was found.

Consumption of MT by aerobic and anaerobic suspensions of T. thioparus strain E6. An aerobic suspension of intact *T. thioparus* strain E6 removed MT from the headspace of a sealed tube at a rate of 27.8 $\text{nmol min}^{-1} (\text{mg cell-protein})^{-1}$, compared with a rate of 6.8 by anaerobic suspension and 2.1 in the absence of organisms under anaerobic conditions (Fig. 2b). No H_2S was detected (i.e. <0.01 μmol in 15 ml) in the gas phase of either the aerobic or anaerobic incubations.

Oxidation by other thiols, homologues of DMDS and inorganic sulphur compounds by suspensions of T. thioparus strain E6. Suspensions of DMDS-grown organisms in the oxygen electrode oxidized MT (5 μM) at 538 $\text{nmol O}_2 \text{ consumed min}^{-1} (\text{mg protein})^{-1}$, but oxidized ethanethiol, 1-butanethiol, 1-hexanethiol, 2-mercaptoethanol and thiophenol (each at 10 μM) at only 78, 62, 57, 21, and 20 $\text{nmol O}_2 \text{ min}^{-1} (\text{mg protein})^{-1}$, respectively. Ethanethiol (0.5 mM), added to a batch culture on 2 mM-DMDS, completely prevented growth.

DMDS (20 μM) was oxidized at 309 $\text{nmol O}_2 \text{ min}^{-1} (\text{mg protein})^{-1}$ compared with only 73 and 20 for diethyl disulphide and dipropyl disulphide (at 20 μM), respectively.

Thiocyanate (50 μM) was not oxidized by DMDS-grown organisms. Sulphide, thiosulphate or tetrathionate (each at 50 μM) were, however, oxidized by DMDS-grown organisms at rates of 291, 120 and 106 $\text{min}^{-1} (\text{mg protein})^{-1}$ respectively. Thiosulphate oxidation was inhibited 50% by FCCP at 0.8 μM or NEM at 9 μM . HQNO (at 150 μM) had no significant effect on thiosulphate oxidation, but tetrathionate oxidation was inhibited 50% by 35 μM -HQNO. Thiosulphate-grown organisms could not oxidize DMDS.

Inhibition of growth on DMDS or thiosulphate by 3-amino-1,2,4-triazole. This inhibitor of catalase severely inhibited growth on DMDS when added at concentrations of 1 mM and above. Growth on thiosulphate was relatively insensitive by comparison (Fig. 3).

Comparison of biomass production in steady state chemostat cultures (D 0.076 h^{-1}) growing on DMDS or DMDS + DMS as limiting substrates. A culture growth-limited by DMDS (1.6 mM) had a steady state biomass content of 23.0 $\text{mg cell-carbon l}^{-1}$, indicating a yield of 14.4 $\text{g cell-carbon (mol DMDS)}^{-1}$. Supplementing the substrate feed to the culture with DMS (1.2 mM) produced an increase in steady state biomass from 23.0 to 37.2 $\text{mg cell-carbon l}^{-1}$. Assuming

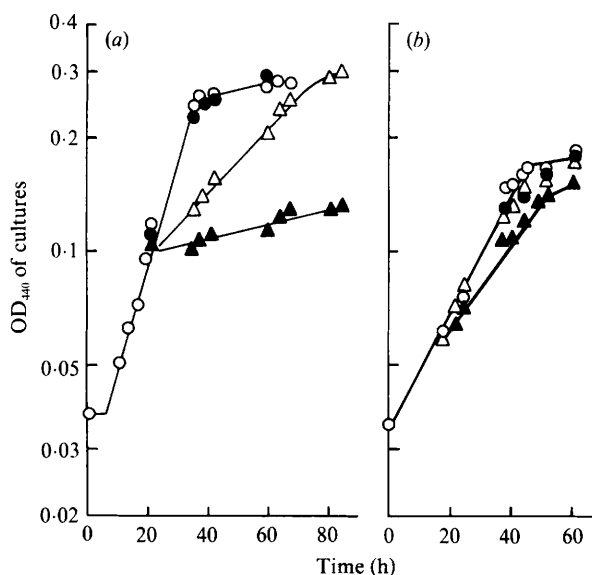


Fig. 3. Effect of 3-amino-1,2,4-triazole on the growth of *T. thioparus* strain E6 on (a) 2 mM-DMDS and (b) 10 mM-thiosulphate. Replicate batch cultures in shaken flasks were supplemented with 3-amino-1,2,4-triazole at 19 h, to give concentrations of 0 (○), 0.1 mM (●), 1.0 mM (△) and 5 mM (▲).

additive biomass production from the DMDS + DMS provided, the yield on DMS could be calculated as $Y_{\text{DMS}} = (37.2 - 23.0)/2 = 11.8$. The difference in yield of 2.6 thus corresponds to the energy made available for growth from the oxidation of the second sulphide group in DMDS. By comparison, the increase in biomass produced by supplying a DMDS-limited culture with thiosulphate (which is energetically equivalent to sulphide) corresponded to a thiosulphate-dependent yield of 2.45 g cell carbon (mol thiosulphate)⁻¹.

Cultures grown separately on DMDS or DMS will not oxidize the other compound, but both were oxidized by organisms removed from the chemostat subject to dual substrate limitation. DMDS (5 μM) was oxidized at 240 nmol O₂ min⁻¹ (mg protein)⁻¹ and DMS at 95.5 (5 μM) and 79 (20 μM).

DISCUSSION

Our results demonstrate that the oxidation of DMDS by *T. thioparus* strain E6 proceeds by a pathway that is analogous to that reported for the oxidation of methylated sulphides by hypomicrobia and *T. thioparus* strain TK-m. The initial step is novel, and must clearly be the reductive cleavage of DMDS to produce two molecules of MT. This reaction is oxygen-independent, being demonstrable under anaerobic conditions. This 'DMDS reductase' used NADH as a reductant when assayed in permeabilized cells, but has not yet been demonstrated in cell-free extracts. The MT so produced is believed to be oxidized via formaldehyde and formate to CO₂ and sulphate (Fig. 4). Although the MT oxidase has not been purified, MT oxidation by crude extracts showed a K_m of about 10 μM , like that reported for the purified enzyme from *Hyphomicrobium* EG (Suylen *et al.*, 1987). Substrate inhibition by MT was also similar, exhibiting a very low K_i value. Failure of the organism to produce H₂S from MT anaerobically indicated the oxygen-requiring nature of the first step of MT metabolism: since H₂S would be expected as a product of an oxygen-requiring oxidase, or of an oxygen-independent hydrolytic cleavage under anaerobic conditions, and under aerobic conditions there would be immediate further oxidation of H₂S, the absence of anaerobic accumulation of H₂S indicates the importance *in vivo* of the MT oxidase.

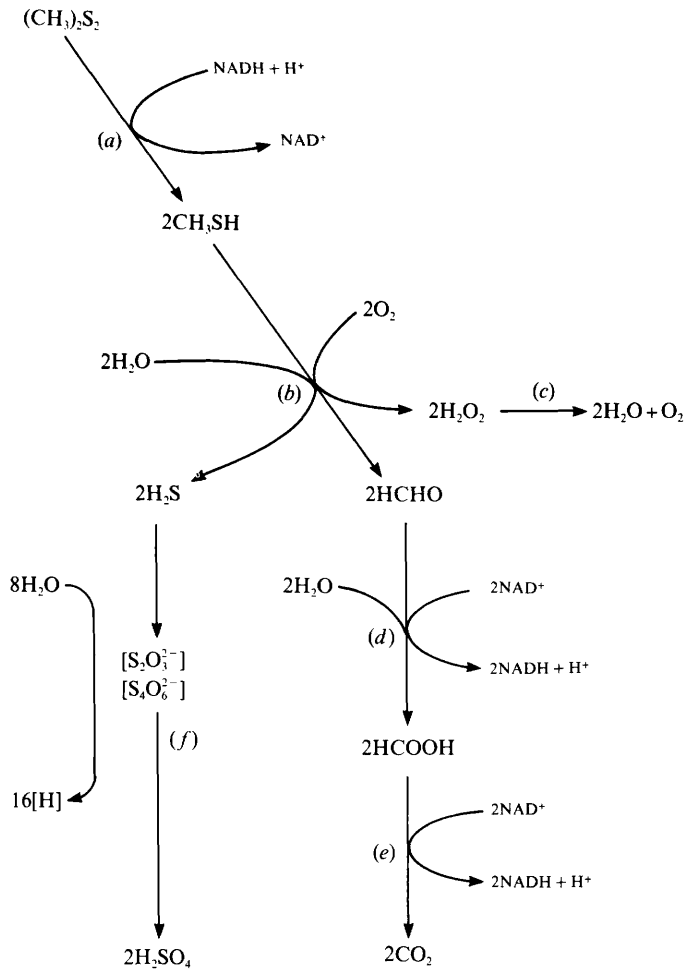


Fig. 4. Proposed mechanism of oxidation of DMDS by *T. thioparus* strain E6. The steps identified are (a) NADH-stimulated DMDS reductase; (b) MT oxidase, producing H_2S and H_2O_2 ; (c) catalase: this is the site of inhibitory action by 3-amino-1,2,4-triazole; (d) NAD-specific formaldehyde dehydrogenase; (e) NAD-specific formate dehydrogenase; (f) sulphide-oxidizing system, possibly involving thiosulphate and tetrathionate as intermediates, and liberating 16 reducing equivalents (from the water used to supply oxygen for sulphate formation), which can be oxidized by 4 mol O_2 via the respiratory chain.

The oxidation of MT by MT oxidase, by analogy with other organisms (De Bont *et al.*, 1981; Suylen *et al.*, 1987), produced H_2O_2 which would have required destruction by catalase. The presence of very high levels of catalase, and the inhibition by the catalase inhibitor 3-amino-1,2,4-triazole of growth on DMDS (but much less so on thiosulphate), both indicate that this step was a central one for MT oxidation.

The results also confirm the autotrophic nature of growth on DMDS, with ribulose biphosphate carboxylase as the key assimilatory enzyme. The specific activity of this enzyme in cell-free extracts was almost double that observed with permeabilized whole cells, indicating ample enzyme to support growth at the rate observed.

The growth yields calculated for DMDS (14.4), DMS (11.8) and thiosulphate (2.45) demonstrate that energy was conserved from both the oxidation of the methyl carbons and the sulphide moieties of DMDS, as was reported for DMS oxidation by *T. thioparus* strain TK-m (Kanagawa & Kelly, 1986). By arithmetic difference in yields the methyl carbons of DMDS

supported a yield of about 9.5 g cell-carbon per two methyl groups oxidized, under the growth conditions used. This may be compared with a yield of up to about 6 g cell-carbon (mol formaldehyde)⁻¹ for *T. versutus* growing in formaldehyde-limited chemostat culture at a similar growth rate (Kelly & Wood, 1984). Since no energy would be conserved by *T. thioparus* strain E6 from the oxidation of the methyl groups of DMDS to formaldehyde, all the energy conserved must be from the oxidation of formaldehyde to CO₂, yielding a maximum of four NADH per DMDS consumed. Since DMDS reduction initially requires one NADH, only three could be available for energy conservation through oxidative phosphorylation. In comparison with *T. versutus*, this would indicate a possible yield of about 9, in reasonable agreement with the experimental estimate of 9.5.

Sulphide oxidation to sulphate was not further characterized, but the oxidation of thiosulphate and tetrathionate by DMDS-grown organisms could indicate their involvement as intermediates in sulphide oxidation. Inhibition of thiosulphate oxidation by FCCP and NEM could indicate the roles of both energy-dependent and membrane-associated enzymes, and an NEM-sensitive tetrathionate oxidation system. The effects of HQNO suggest that electron transport between cytochromes *b* and *c* occurred during tetrathionate oxidation but not during the oxidation of thiosulphate to tetrathionate, as also occurs in *T. tepidarius* (Lu & Kelly, 1988).

We thank the Natural Environment Research Council for supporting this work.

REFERENCES

- BEJI, A., IZARD, D., GAVINI, F., LECLERC, H., LESEINE-DELSTANCHE, M. & KREMBEL, J. (1987). A rapid chemical procedure for isolation and purification of chromosomal DNA from Gram-negative bacilli. *Analytical Biochemistry* **162**, 18–23.
- DE BONT, J. A. M., VAN DIJKEN, J. P. & HARDER, W. (1981). Dimethyl sulphoxide and dimethyl sulphide as a carbon, sulphur and energy source for growth of *Hyphomicrobium* S. *Journal of General Microbiology* **127**, 315–323.
- DISPIRITO, A. A., LOH, W. H.-T. & TUOVINEN, O. H. (1983). A novel method for the isolation of bacterial quinones and its application to appraise the ubiquinone composition of *Thiobacillus ferrooxidans*. *Archives of Microbiology* **135**, 77–80.
- FREDERICQ, E., OTH, A. & FONTAINE, F. (1961). The ultraviolet spectrum of deoxyribonucleic acids and their constituents. *Journal of Molecular Biology* **3**, 11–17.
- HAYWOOD, G. W. & LARGE, P. J. (1981). Microbial oxidation of amines. Distribution, purification and properties of two primary amine oxidases from the yeast *Candida boidinii* grown on amines as sole nitrogen source. *Biochemical Journal* **199**, 187–201.
- KANAGAWA, T. & KELLY, D. P. (1986). Breakdown of dimethyl sulphide by mixed cultures and by *Thiobacillus thioparus*. *FEMS Microbiology Letters* **34**, 13–19.
- KANAGAWA, T., DAZAI, M. & FUKUOKA, S. (1982). Degradation of *O,O*-dimethyl phosphorodithioate by *Thiobacillus thioparus* TK-1 and *Pseudomonas* AK-2. *Agricultural and Biological Chemistry* **46**, 2571–2578.
- KELLY, D. P. & HARRISON, A. P. (1988). *Thiobacillus*. In *Bergey's Manual of Systematic Bacteriology*, vol. 3. Edited by M. P. Bryant, N. Pfennig & J. T. Staley. Baltimore: Williams & Wilkins (in the Press).
- KELLY, D. P. & WOOD, A. P. (1982). Autotrophic growth of *Thiobacillus* A2 on methanol. *FEMS Microbiology Letters* **15**, 229–233.
- KELLY, D. P. & WOOD, A. P. (1984). Potential for methylotrophic autotrophy in *Thiobacillus versutus* (*Thiobacillus* sp. strain A2). In *Microbial Growth on C₁ Compounds*, pp. 324–329. Edited by R. L. Crawford & R. S. Hanson. Washington, DC: American Society for Microbiology.
- KELLY, D. P., CHAMBERS, L. A. & TRUDINGER, P. A. (1969). Cyanolysis and spectrophotometric estimation of trithionate in mixture with thiosulfate and tetrathionate. *Analytical Chemistry* **41**, 898–901.
- LU, W.-P. & KELLY, D. P. (1988). Kinetic and energetic aspects of inorganic sulphur compound oxidation by *Thiobacillus tepidarius*. *Journal of General Microbiology* **134**, 865–876.
- MERCK INDEX (1968). 8th Edition. Edited by P. G. Stecher. Rahway, NJ: Merck & Co.
- PATEL, R. N. & HOARE, D. S. (1971). Physiological studies of methane and methanol oxidizing bacteria: oxidation of C-1 compounds by *Methylococcus capsulatus*. *Journal of Bacteriology* **107**, 187–192.
- SMITH, N. A. & KELLY, D. P. (1988). Isolation and physiological characterization of autotrophic sulphur bacteria oxidizing dimethyl disulphide as sole source of energy. *Journal of General Microbiology* **134**, 1407–1417.
- STIRLING, D. I. & DALTON, H. (1978). Purification and properties of an NAD(P)⁺-linked formaldehyde dehydrogenase from *Methylococcus capsulatus* (Bath). *Journal of General Microbiology* **107**, 19–29.
- SUYLEN, G. M. H., STEFESS, G. C. & KUENEN, J. G. (1986). Chemolithotrophic potential of a *Hyphomicrobium* species, capable of growth on methylated sulphur compounds. *Archives of Microbiology* **146**, 192–198.
- SUYLEN, G. M. H., LARGE, P. J., VAN DIJKEN, J. P. & KUENEN, J. G. (1987). Methyl mercaptan oxidase, a key enzyme in the metabolism of methylated sulphur compounds by *Hyphomicrobium* EG. *Journal of General Microbiology* **133**, 2989–2997.

TUOVINEN, O. H. & KELLY, D. P. (1973). Studies on the growth of *Thiobacillus ferrooxidans*. *Archiv für Mikrobiologie* **88**, 285–298.

ULITZUR, S. (1972). Rapid determination of DNA base composition by ultraviolet spectroscopy. *Biochimica et biophysica acta* **272**, 1–11.

WOOD, A. P. & KELLY, D. P. (1985). Physiological characteristics of a new thermophilic obligately chemolithotrophic *Thiobacillus* species, *Thiobacillus tepidarius*. *International Journal of Systematic Bacteriology* **35**, 434–437.