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Thiosulfate Oxidation and Tetrathionate Reduction by Intact Cells of Marine Pseudomonad Strain 16B

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Levels of thiosulfate-oxidizing enzyme (TSO) and tetrathionate reductase (TTR) were measured in washed cell suspensions of a heterotrophic marine thiosulfate-oxidizing bacterium, strain 16B. TSO activity remained virtually constant in aerobically and anaerobically grown cells and was unaffected by the presence or absence of thiosulfate and tetrathionate in the growth medium. TTR was also present in cells grown aerobically and anaerobically, but its activity was threefold greater in cells cultured in media containing tetrathionate or thiosulfate. Tetrathionate appears to be the inducer of increased TTR activity in both aerobically and anaerobically grown cells. TTR (constitutive or induced) and TSO have different pH and temperature optima. Both TTR activities were unaffected by 10 mM KCN, which reversed oxygen inhibition of tetrathionate reduction. TSO was partially inhibited by 5 μM KCN and completely inhibited by 90 μM KCN. These findings and results of experiments to determine the influence of several inorganic electron donors and acceptors on TSO and TTR activities suggest that constitutive TSO and TTR represent reverse activities of the same enzyme, whereas inducible TTR is a separate enzyme used by strain 16B only for anaerobic respiration of tetrathionate. The bacterium appears well adapted to growth in environments characterized by low oxygen tension, dilute organic carbon concentrations, and the presence of a variety of reduced, inorganic sulfur compounds.

Marine pseudomonad strain 16B metabolizes a variety of inorganic sulfur compounds. This facultatively anaerobic heterotroph, originally isolated from sulfide-bearing water near the oxygen-sulfide interface of the Black Sea (22, 23), oxidizes thiosulfate to tetrathionate under aerobic conditions (21), a process which increases its growth rate in the presence of an organic carbon and energy source. Under anaerobic conditions, tetrathionate, thiosulfate, sulfite (24), and trithionate (J. H. Tuttle and H. W. Jannasch, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, N9, p. 186) are terminal electron acceptors for anaerobic respiration and growth.

Bacterial oxidation of thiosulfate to tetrathionate is catalyzed by thiosulfate-oxidizing enzyme (TSO). This enzyme is widespread among the thiobacilli (13, 18, 20, 27) and has been implicated in energy generation by these chemolithotrophs chiefly at high concentrations of thiosulfate (8). TSO has also been demonstrated in a variety of heterotrophic bacteria (3, 14, 15, 17, 19, 26), but except in certain marine heterotrophs (21), its physiological function in heterotrophic microorganisms is unclear. However, tetrathionate reductase (TTR), which catalyzes the reduction of tetrathionate to thiosulfate (i.e., the reverse of TSO), has so far been found only in heterotrophic bacteria. The presence of TTR may be used as a taxonomic tool for identification of certain *Enterobacteriaceae* (7, 10), and TTR is commonly present in *Salmonella*, *Citrobacter*, and *Proteus* (7, 9, 10). Representatives of all three genera have been demonstrated to utilize tetrathionate as a terminal electron acceptor for anaerobic respiration during carbohydrate metabolism (4, 6, 16).

Neither thiobacilli nor *Enterobacteriaceae* have been shown to possess both TSO and TTR activities. I know of only two reports demonstrating the activities of both enzymes in the same bacterium: two soil isolates described by Trudinger (19) and several of our own marine isolates (21, 24). In this paper, I report evidence which demonstrates constitutive TSO activity and suggests two different TTR activities, one constitutive and the other inducible, in washed, whole cells of marine pseudomonad strain 16B.

MATERIALS AND METHODS

Bacteria, media, and culture conditions. Strain 16B was maintained on thiosulfate-seawater medium as previously described (22).

Mass cultures were prepared in the defined medium of Tuttle and Jannasch (24) with 12 mM sodium pyruvate as the carbon and energy source. When used,

sodium thiosulfate or potassium tetrathionate was added at a 10 mM concentration. The initial pH of the medium was adjusted to 7.5 with NaOH and filter sterilized (0.22-μm membranes; Millipore Corp.).

For aerobic cultures, the medium was aseptically transferred to a 1-liter reaction kettle fitted with a gas dispersion tube. After the addition of a 4% (vol/vol) inoculum from a 24-h preculture prepared in the same medium, the culture was aerated with sterile air and continuously stirred with a magnetic stirring bar for 24 h (absorbance at 450 nm, approximately 0.8) at 24 ± 2°C before harvesting.

For anaerobic cultures, the filter-sterilized medium was aseptically transferred to a sterile 2-liter dispensing bottle, gassed out with O_2-free N_2 (heated copper column) for 1 h, and dispensed anaerobically into a gassed-out 1-liter reaction kettle. After addition of a 4% (vol/vol) inoculum from a 48-h anaerobic preculture prepared in the same medium, the culture was continuously sparged with O_2-free N_2 gas and stirred as above for 48 h (absorbance at 450 nm, approximately 0.25) at 24 ± 2°C.

Cells were harvested by centrifugation at 10,000 × *g* and 4°C, washed twice with sterile artificial seawater (pH 8; Seven Seas Marine Mix, Utility Chemical Co.), and resuspended in 10 ml of sterile artificial seawater. Concentrated cell suspensions were stored at 4°C for no longer than 4 days. Storage had no effect on TSO or TTR activity.

Dry weights. Portions (100 ml each) of aerobically and anaerobically grown cultures were dried at 98°C to a constant weight corrected for solids present in the culture filtrates. Absorbance curves (450 nm) were determined by appropriate dilution of the same cultures. An absorbance of 1.00 (1-cm cuvette) corresponded to 407 μg (dry weight) of bacteria per ml.

Measurement of TSO activity. Thiosulfate oxidation was estimated from oxygen uptake rates determined polarographically with a Clarke-type electrode (Yellow Springs Instrument Co.) fitted with a 0.001-in. (ca. .0254 mm) Teflon membrane and connected to a Microcord 44 recorder (Photovolt Corp.). Temperature was controlled (37°C, unless stated otherwise) in a 3.5-ml jacketed reaction vessel. Reaction mixtures consisted of 0.1 M phosphate–2% NaCl buffer (pH 6.5, unless indicated otherwise), 20 mM $Na_2S_2O_3$, and 0.05 mg of chloramphenicol per ml. Measurements were started by injection of a 25-μl portion (~2 mg) of temperature-equilibrated cell suspension through a small hole in the top of the reaction vessel. All reagents were air saturated, and the experimental rates, normally determined over a 2-min incubation period, were corrected for endogenous respiration (thiosulfate omitted). Oxygen uptake rates were converted to thiosulfate oxidation rates on the basis that the oxidation of 1.0 mol of thiosulfate by strain 16B consumes 0.25 mol of O_2 (21).

Measurement of TTR activity. Tetrathionate reduction was measured with a modified method similar to that described by Kaprálek and Pichinoty (5). The reaction mixture contained 0.50 mg of chloramphenicol, 200 μmol of sodium pyruvate, and 20 mg (dry weight) of washed cells per 10-ml total volume of 0.1 M phosphate–2% NaCl buffer (pH 7.5, unless stated otherwise). The mixture was placed in an 18-mm culture tube held at the desired temperature (37°C, unless stated otherwise) in a water bath and continuously gassed with O_2-free N_2 delivered with a Pasteur pipette extending to the bottom of the tube. The reaction was started by adding 250 μmol of $K_2S_4O_6$. At suitable time intervals, 2.0-ml samples were rapidly transferred to 125-ml flasks containing 2 ml of 10% (vol/vol) acetic acid, and the thiosulfate formed was determined by iodometric titration. Titration values were corrected with control mixtures prepared without added tetrathionate. Since strain 16B does not metabolize thiosulfate anaerobically until tetrathionate is completely consumed, titration values are stoichiometrically related to the quantity of tetrathionate reduced (24). Unless indicated otherwise, rates were determined from regression plots of titration values obtained between 10 and 40 min of incubation.

Chemicals and chemical determinations. All commercial chemicals were of reagent quality. Tetrathionate and trithionate were synthesized by procedures described by Roy and Trudinger (12) and checked for purity by ascending paper chromatography as previously described (21, 24). The pH was determined with a Metrohm expanded scale pH meter (Brinkmann Instruments Inc.).

RESULTS

Influence of culture conditions on TSO and TTR activities. In agreement with earlier observations (21), TSO is a constitutive enzyme in strain 16B. It was found at approximately the same specific activity whether or not reduced sulfur was supplied in the medium and under aerobic or anaerobic growth conditions (Table 1). However, TTR activity was about threefold greater when cultures were grown with thiosulfate or tetrathionate. The inducer for increased TTR activity is probably tetrathionate, the product of thiosulfate oxidation by strain 16B under aerobic growth conditions (21).

It is not likely that residual TTR activity present in cells cultured aerobically without reduced sulfur (Table 1) is due to carry-over of trace amounts of tetrathionate or thiosulfate with inocula from thiosulfate-seawater medium

TABLE 1. *Effect of culture conditions on activities of TSO and TTR*

Culture conditions	TSO activity[a]	TTR activity
Aerobic, no reduced S	48.2 ± 3.0	5.3 ± 0.9
Aerobic, 20 mM $S_2O_3^{2-}$	52.7 ± 0.9	16.4 ± 0.2
Aerobic, 10 mM $S_4O_6^{2-}$	53.8 ± 1.5	16.0 ± 0.5
Anaerobic, 10 mM $S_4O_6^{2-}$	53.6 ± 2.4	14.8 ± 1.0

[a] Results are expressed as micromoles of thiosulfate oxidized (TSO) or formed from tetrathionate (TTR) per hour per milligram (dry weight) of cells. Each value represents the average of six runs obtained with cell suspensions from two different cultures prepared under each culture condition. The variance values represent one standard deviation from the mean.

stock cultures. Three successive transfers were made in precultures (thiosulfate and tetrathionate absent) before mass cultures used to obtain the test cell suspensions were inoculated. Rather, the results suggest a constitutive TTR which may be associated with TSO activity. Although strain 16B is capable of pyruvate fermentation under anaerobic conditions in the absence of exogenously supplied electron acceptors, growth is sparse (24). Under the culture conditions used in the present work, I was unable to obtain sufficient quantities of cells from pyruvate fermentation cultures to obtain TTR data. However, bacteria cultured in this manner are capable of thiosulfate oxidation. Cultures grown aerobically yielded at least three times as much dry weight of cells as did cultures grown anaerobically with tetrathionate as the terminal electron acceptor.

Effect of temperature, pH, and thiosulfate concentration on TSO activity. Maximum thiosulfate oxidation occurred within the range of 32 to 37°C (Fig. 1). At 13°C, a temperature which is more indicative of the Black Sea water from which strain 16B was originally isolated (23), the velocity of thiosulfate oxidation was still about half the maximum. TSO activity was destroyed by heating at 60°C for 15 min. The pH optimum for thiosulfate oxidation was in the range of 6.0 to 6.5 (Fig. 2). This is consistent with previously reported manometric determinations and corresponds to the pH range of maximal growth rate stimulation of strain 16B by thiosulfate (21). However, the optimal growth pH (7.5 to 8.0) for strain 16B in the absence of thiosulfate in aerobic cultures is significantly higher (21).

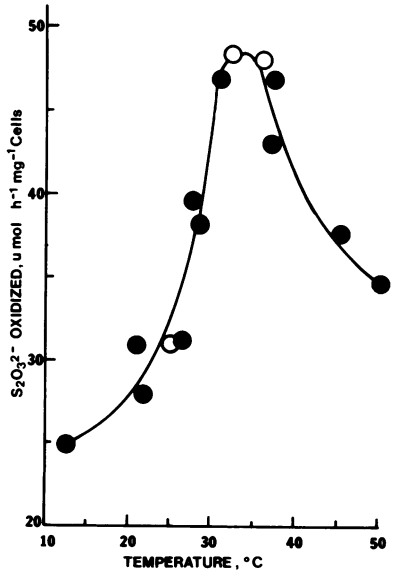

FIG. 1. *Influence of temperature on the rate of thiosulfate oxidation at pH 6.5. Symbols: ●, cells grown aerobically without reduced sulfur compounds; ○, cells grown aerobically with 20 mM* $S_2O_3^{2-}$.

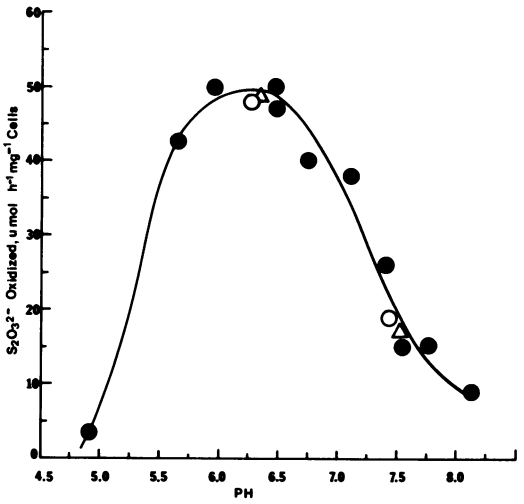

FIG. 2. *Influence of pH on the rate of thiosulfate oxidation at 37°C. Symbols: ●, cells grown aerobically without reduced sulfur compounds; ○, cells grown aerobically with 20 mM* $S_2O_3^{2-}$; △, *cells grown anaerobically with 10 mM* $S_4O_6^{2-}$.

The K_m apparent for thiosulfate was determined at pH 6.25 and 37°C for two different cell suspensions cultured aerobically in the presence or absence of thiosulfate. From Eadie-Hofstee and Lineweaver-Burke plots, the K_m apparent was 153 ± 8 μM thiosulfate, about 10-fold lower than the value determined by manometric methods at pH 6.5 and 25°C (21).

Effect of temperature, pH, and tetrathionate concentration on TTR activity. The temperature optimum of TTR activity for cells grown in the absence of reduced sulfur compounds was about 37°C (Fig. 3). Maximum rates for tetrathionate reduction in thiosulfate- or tetrathionate-grown cells also occurred at 37°C. These data are not plotted because rates below 30°C or above 40°C were not determined. Regardless of cell culture conditions, incubation for more than 15 min at temperatures exceeding 40°C resulted in cell lysis and destruction of TTR activity measurable with the procedure used. Except for the abrupt decrease in TTR activity of thiosulfate-grown cells at pH 6.0, the response of TTR activity to pH in cells grown in the presence or absence of thionates was similar (Fig. 4). The pH optimum was about 7.5, and 70% of maximal activity remained at pH 6.5.

I was unable to determine a K_m value for

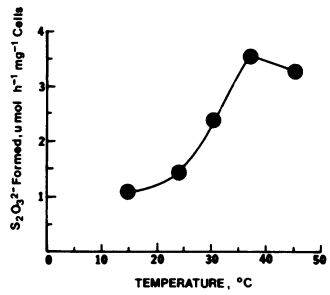

FIG. 3. *Influence of temperature on the rate of tetrathionate reduction at pH 6.5 by cells grown aerobically without reduced sulfur compounds.*

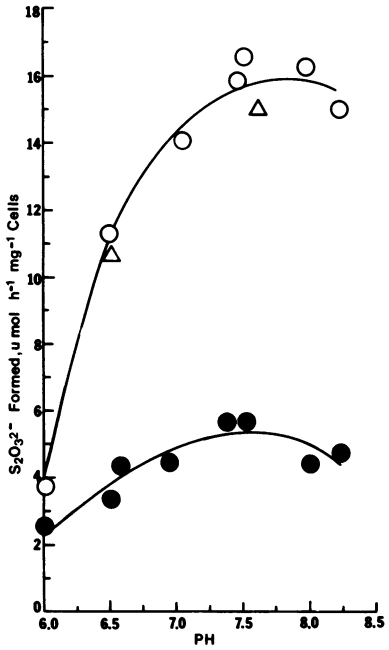

FIG. 4. *Influence of pH on the rate of tetrathionate reduction at 37°C. Symbols: ●, cells grown aerobically without reduced sulfur compounds; ○, cells grown aerobically with 20 mM $S_2O_3^{2-}$; △, cells grown anaerobically with 10 mM $S_4O_6^{2-}$.*

tetrathionate due to the inability to reproducibly determine low concentrations of thiosulfate formed at low initial tetrathionate concentrations (<2 mM). Kinetic determinations will need to be done with cell-free enzyme and a different assay procedure. However, 25 mM tetrathionate used in the experiments reported here is clearly saturating. At the maximum rate of tetrathionate reducion (about 8 μmol of $S_4O_6^{2-}$ h^{-1} mg of $cells^{-1}$, which is equivalent to 16 μmol of $S_2O_3^{2-}$ formed h^{-1} mg of $cells^{-1}$ in my assay procedure), the progress curves were linear for at least 60 min of incubation. On this basis, 9 mM tetrathionate is probably still saturating, and the K_m for tetrathionate must be approximately 4.5 mM or lower.

Effect of oxygen and cyanide. When tetrathionate was added to reaction mixtures bubbled with nitrogen, tetrathionate reduction began immediately (Fig. 5). When the mixtures were bubbled with air instead of nitrogen, thiosulfate was not formed, but tetrathionate reduction began immediately at a constant rate when nitrogen replaced air. These results demonstrate reversible inhibition of TTR activity by oxygen.

Experiments were conducted to determine whether the inhibition of the terminal oxidase coupling thiosulfate oxidation to oxygen reduction could prevent oxygen inhibition of TTR activity. KCN was a potent inhibitor of TSO activity, causing 50% inhibition of thiosulfate oxidation at 8 μM and complete inhibition at 100 μM (Fig. 6). TTR was virtually insensitive to KCN until the KCN concentration exceeded 25 mM, which is equal to the concentration of tetrathionate used in the reaction mixture. Even at 30 mM KCN, TTR activity was inhibited by not more than 10%, independent of cell culture conditions. KCN reacts abiologically with tetrathionate and, to a lesser extent, with thiosulfate under the experimental conditions used. This alone could account for the inhibition of TTR activity by KCN concentrations of 30 mM or higher; i.e., TTR activity may have been decreased by chemical depletion of the substrate. The product of the chemical reaction, KSCN, had a negligible effect on thiosulfate

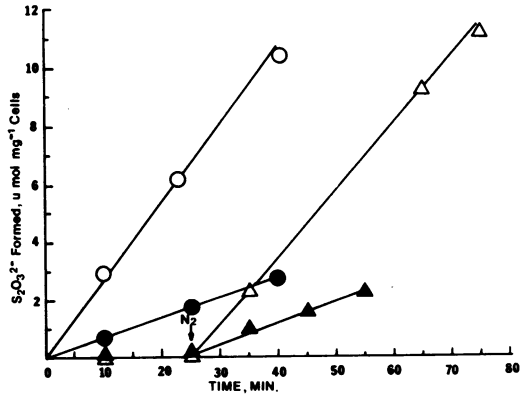

FIG. 5. *Reversal of oxygen inhibition of tetrathionate reduction by nitrogen. Symbols: ●, cells grown aerobically without reduced sulfur compounds, reaction mixtures bubbled with nitrogen; ○, cells grown aerobically with 20 mM $S_2O_3^{2-}$, reaction mixtures bubbled with nitrogen; ▲, cells grown aerobically without reduced sulfur compounds, reaction mixtures aerated for 25 min; △, cells grown aerobically with 20 mM $S_2O_3^{2-}$, reaction mixtures aerated for 25 min.*

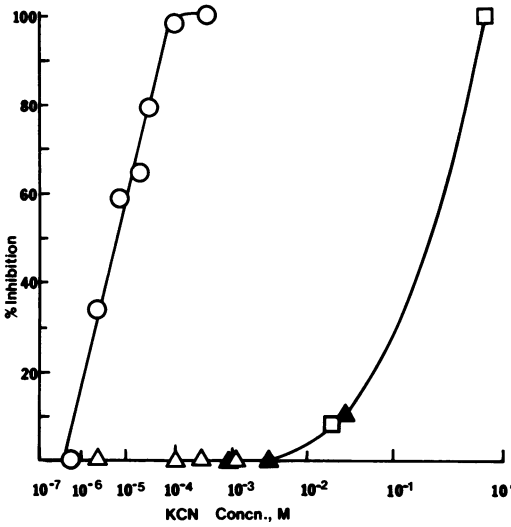

FIG. 6. *Inhibition of TSO and TTR activities by cyanide. Symbols: ○, TSO, cells grown aerobically with 20 mM $S_2O_3^{2-}$; ▲, TTR, cells grown aerobically without reduced sulfur compounds; △, TTR, cells grown aerobically with 20 mM $S_2O_3^{2-}$; □, TTR, cells grown anaerobically with 10 mM $S_4O_6^{2-}$.*

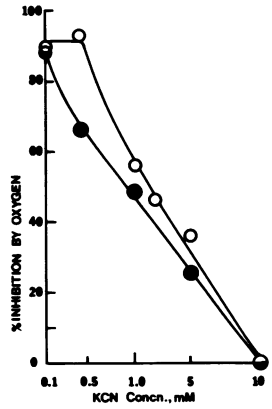

FIG. 7. *Ability of cyanide at different concentrations to reverse oxygen inhibition of tetrathionate reduction. Controls were reaction mixtures aerated in the absence of KCN and represent 100% inhibition (tetrathionate not reduced). Symbols: ■, cells grown aerobically without reduced sulfur compounds, 0.81 mg (dry weight) per ml; ○, cells grown aerobically with 20 mM $S_2O_3^{2-}$, 1.84 mg (dry weight) per ml.*

oxidation (20 mM KSCN) and tetrathionate reduction (25 mM KSCN) when added to reaction mixtures.

KCN prevented reversible inhibition of tetrathionate reduction by oxygen (Fig. 7). In these experiments, reaction mixtures were vigorously bubbled with air in the presence of various concentrations of KCN, followed by the addition of tetrathionate and subsequent analysis for thiosulfate at appropriate time intervals. Much higher concentrations of cyanide were required to restore TTR activity (Fig. 7) than to completely inhibit thiosulfate oxidation (Fig. 6); e.g., 100 μM KCN had only a slight restorative influence on terathionate reduction, and 10 mM KCN was required to permit an uninhibited rate of tetrathionate reduction in the presence of air. This observation indicates that strain 16B may possess more than one terminal oxidase coupled to oxygen; i.e., pyruvate oxidation and thiosulfate oxidation may occur via different electron transport chains during aerobic metabolism. Alternatively, the differing results could be due simply to the approximately threefold-greater cell concentrations used in the tetrathionate reduction assay, as compared with those used in thiosulfate oxidation measurements.

The ability of KCN to restore tetrathionate reduction in the presence of oxygen differed with cell culture conditions (Fig. 7). TTR activity in cells cultured with thiosulfate or tetrathionate was slightly more sensitive to oxygen than was TTR in cells grown in the absence of reduced sulfur compounds. This effect may be related to the use of inducible TTR during anaerobic respiration and growth of the bacterium under anaerobic conditions (24).

Figure 8 shows the results of an experiment in which aerated cell suspensions were incubated with tetrathionate before the addition of 10 mM cyanide. Tetrathionate reduction appeared to begin rapidly when cyanide was added, even though vigorous aeration was continued. The reason for the apparently stimulated rates of tetrathionate reduction between 20 and 30 min is not clear, and the linear rates between 30 and 50 min (9.0 μmol h^{-1} mg^{-1} and 3.6 μmol h^{-1} mg^{-1} for induced and uninduced cells, respectively) were somewhat lower than rates for cell suspensions bubbled with N_2 under otherwise similar experimental conditions.

Effect of potential electron donors and acceptors on TSO and TTR activities. Nitrate and a variety of reduced, inorganic sulfur compounds were tested for their ability to inhibit thiosulfate oxidation and tetrathionate reduction (Table 2). TSO activity was inhibited strongly by sulfite, was inhibited less strongly by trithionate, and was unaffected by nitrate and tetrathionate. The absence of product inhibition by tetrathionate agrees with manometric experiments previously reported (21).

TTR activity in uninduced cells was inhibited fairly strongly by sulfite and moderately by trithionate, but was inhibited only slightly by nitrate. Thiosulfate caused a substantial product inhibition of TTR in uninduced cells. In con-

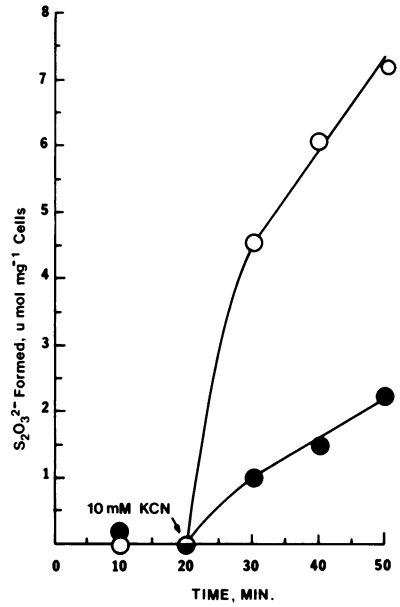

FIG. 8. *Ability of KCN to rapidly reverse oxygen inhibition of tetrathionate reduction. Reaction mixtures were continuously aerated throughout the incubation period. Symbols: ●, cells grown aerobically without reduced sulfur compounds; ○, cells grown aerobically with 20 mM $S_2O_3^{2-}$.*

TABLE 2. *Effect of potential electron donors and acceptors on TSO and TTR activities*

Donor or acceptor added	% Inhibition of activity[a]		
	TSO	TTR[b]	TTR[c]
$K_2S_4O_6$	0		
$Na_2S_2O_3$		60	0
Na_2SO_3	95	57	5
$K_2S_3O_6$	59	25	0
KNO_3	0	11	0

[a] Percent inhibition values are compared to rates with 20 mM thiosulfate alone (TSO) or 25 mM tetrathionate alone (TTR). Donors and acceptors were added at a concentration equal to the substrate. Values for TTR were corrected for reactivity of the added acceptor in the titration measurements. Duplicate runs varied by no more than 5% inhibition.

[b] Cells grown without reduced sulfur compounds.

[c] Cells grown with 20 mM $S_2O_3^{2-}$ or 10 mM $S_4O_6^{2-}$.

trast, TTR activity in cells grown with tetrathionate or thiosulfate was virtually unaffected by any of the compounds tested.

The difference in the effect of thiosulfate on tetrathionate reduction by cells grown with or without thiosulfate or tetrathionate is shown graphically in Fig. 9. In reaction mixtures containing induced cells, thiosulfate had no effect when added 10 min before the addition of tetrathionate (time zero) or 25 min after tetrathionate reduction had begun. In the latter case, the actual thiosulfate concentration upon addition of thiosulfate was 37.5 mM, whereas the tetrathionate concentration had decreased to 18.7 mM, in contrast to the equimolar ratio of tetrathionate to thiosulfate present when thiosulfate was added before tetrathionate.

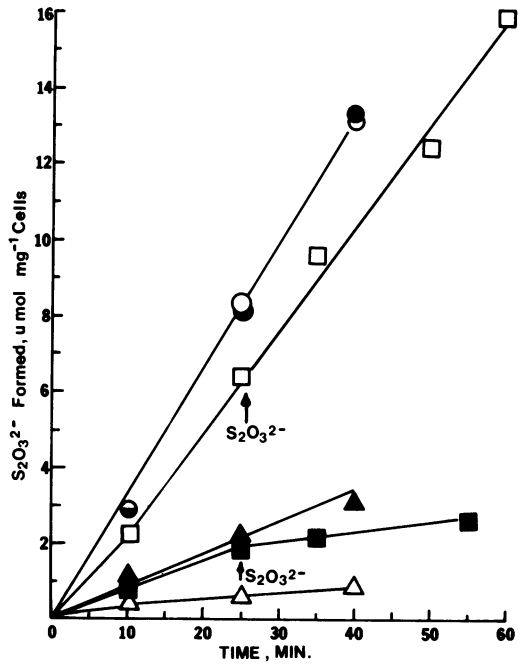

FIG. 9. *Influence of thiosulfate on tetrathionate reduction. Symbols: ●, control, cells grown aerobically with 20 mM $S_2O_3^{2-}$; ○, thiosulfate added at time zero, cells grown aerobically with 20 mM $S_2O_3^{2-}$; ▲, control, cells grown aerobically without reduced sulfur compounds; △, thiosulfate added at time zero, cells grown aerobically without reduced sulfur compounds; ■, thiosulfate added at 25 min of incubation, cells grown aerobically without reduced sulfur compounds; □, thiosulfate added at 25 min of incubation, cells grown anaerobically with 10 mM $S_4O_6^{2-}$.*

In reaction mixtures containing uninduced cells, tetrathionate reduction in the presence of previously added thiosulfate proceeded at about one-third the rate of uninhibited cells. The addition of thiosulfate at 25 min caused an immediate decrease in tetrathionate reduction to a rate equal to that observed in cells pretreated with thiosulfate.

DISCUSSION

Marine pseudomonad strain 16B has been demonstrated to contain a constitutive TSO and two distinct TTR activities, one of which is constitutive and the other of which is inducible

by tetrathionate in the culture medium under aerobic or anaerobic growth conditions. Whether thiosulfate or other reduced sulfur compounds which serve as terminal electron acceptors for anaerobic growth of strain 16B (24) also cause induction of increased TTR activity remains to be investigated.

TTR activity is formed concurrently with TSO under aerobic conditions and in the absence of reduced sulfur compounds in the medium (Table 1). If this enzyme were responsible for anaerobic growth with tetrathionate as the terminal oxidant, it seems unlikely that an inducible TTR would need to be synthesized. TSO and constitutive TTR are inhibited by sulfite and trithionate, whereas inducible TTR is virtually unaffected (Table 2). These observations and the finding that constitutive TTR is inhibited by its product, thiosulfate, whereas TSO is not inhibited by its product, tetrathionate (Table 2), are consistent with the proposal that constitutive TSO and TTR represent the reversible activities of the same enzyme whose major physiological function is the oxidation of thiosulfate in the presence of a suitable electron acceptor. This possibility is also supported by the finding that inducible TTR activity is unaffected by thiosulfate (Table 2 and Fig. 9). Simultaneously inducible TSO and TTR in soil isolate A-50 have been suggested to represent the reversible activity of the same enzyme (19). I emphasize, however, that the proof that constitutive TSO and TTR activities in strain 16B are of the same enzyme must consist of evidence that both thiosulfate oxidation and tetrathionate reduction are catalyzed by the same protein. We have recently reported the isolation and partial purification of TSO from strain 16B (J. H. Schwartz and J. H. Tuttle, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, K148, p. 170) and are currently evaluating techniques to measure TTR and other enzymes involved in anaerobic dissimilation of inorganic sulfur by strain 16B (24) and a variety of other facultatively anaerobic marine bacteria.

The inhibition of tetrathionate reduction in strain 16B by oxygen and the reversal of this inhibition by cyanide or nitrogen (Fig. 5 to 8) agree with studies on TTR activity in *Citrobacter* (5) and *Salmonella* (11), but the formation of both constitutive and inducible TTR activities in aerobically grown strain 16B (Table 1) may be unusual. Synthesis of constitutive TTR in *Proteus mirabilis* (1) and inducible TTRs of *Salmonella* (11) and *Citrobacter* (5) is repressed by oxygen. However, TTR is synthesized with TSO in aerobic cultures of soil bacterium A-50 (19). Its formation has also been reported in shaken *Citrobacter* cultures (5), but this apparent anomaly was explained by the occurrence of low oxygen tension in the medium at high cell densities near the end of the exponential growth phase. In the absence of oxygen measurements in my cultures, which were grown aerobically in the presence of thiosulfate, it cannot be unequivocably stated that inducible TTR is synthesized by strain 16B in the presence of substantial concentrations of oxygen. It seems likely, however, that synthesis of constitutive TTR is unaffected by aerobic growth conditions.

Nitrate had little or no effect on either inducible or constitutive TTR activity (Table 2). This suggests that TTR and nitrate reductase are not identical enzymes in strain 16B. The bacterium has been demonstrated to utilize nitrate as a terminal electron acceptor for anaerobic growth (24). Under anaerobic growth conditions, TTR synthesis is repressed by nitrate in *Salmonella* (11), *Citrobacter* (5), and *Proteus* (1). Whether nitrate represses TTR synthesis in strain 16B has not yet been investigated, but in anaerobic growth experiments similar to those described previously (24), the bacterium oxidizes thiosulfate to tetrathionate during growth in acetate-nitrate or pyruvate-nitrate media (unpublished data). If constitutive TTR and TSO are reversible activities of the same enzyme, then nitrate is not likely to influence synthesis of constitutive TTR.

The ecological role of tetrathionate-reducing bacteria remains virtually uninvestigated. Physiological studies (21, 23, 24) indicate, however, that these facultatively anaerobic microorganisms would be well adapted to conditions of low oxygen tension, dilute nutrient concentrations, and the presence of various reduced sulfur compounds. Bacteria capable of both tetrathionate reduction and thiosulfate oxidation have been directly enriched in media similar to that used in the experiments described here from anoxic marine basins (25), from the oxygen-sulfide interface of a Texas reservoir during summer stratification (unpublished data), and, very recently, from the deep sea environment of the Galapagos rift-vent upwelling, where they apparently coexist with chemolithotrophic sulfur-oxidizing bacteria (2). Experiments are currently being conducted in this laboratory to clarify the ecological relationships existing among heterotrophic thiosulfate-oxidizing and tetrathionate-reducing bacteria, chemolithotrophic sulfur bacteria, and obligately anaerobic sulfate reducers.

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