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Role of a Ferric Ion-Reducing System in Sulfur Oxidation of *Thiobacillus ferrooxidans*

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The properties of a ferric ion-reducing system which catalyzes the reduction of ferric ion with elemental sulfur was investigated with a pure strain of *Thiobacillus ferrooxidans*. In anaerobic conditions, washed intact cells of the strain reduced 6 mol of Fe^{3+} with 1 mol of elemental sulfur to give 6 mol of Fe^{2+}, 1 mol of sulfate, and a small amount of sulfite. In aerobic conditions, the 6 mol of Fe^{2+} produced was immediately reoxidized by the iron oxidase of the cell, with a consumption of 1.5 mol of oxygen. As a result, Fe^{2+} production was never observed under aerobic conditions. However, in the presence of 5 mM cyanide, which completely inhibits the iron oxidase of the cell, an amount of Fe^{2+} production comparable to that formed under anaerobic conditions was observed under aerobic conditions. The ferric ion-reducing system had a pH optimum between 2.0 and 3.8, and the activity was completely destroyed by 10 min of incubation at 60°C. A short treatment of the strain with 0.5% phenol completely destroyed the ferric ion-reducing system of the cell. However, this treatment did not affect the iron oxidase of the cell. Since a concomitant complete loss of the activity of sulfur oxidation by molecular oxygen was observed in 0.5% phenol-treated cells, it was concluded that the ferric ion-reducing system plays an important role in the sulfur oxidation activity of this strain, and a new sulfur-oxidizing route is proposed for *T. ferrooxidans*.

Thiobacillus ferrooxidans has been considered one of the most valuable microorganisms for bacterial leaching of sulfide ores. Its value may be due to an ability to utilize for growth both the Fe^{2+} and the sulfur moieties in sulfide ores. Many investigators have reported on cell growth on reduced sulfur compounds (7, 16, 22), the activities and mechanism of sulfur oxidation (3, 5, 8, 12, 15), and the enzymes involved in sulfur oxidation by *T. ferrooxidans* (13, 14, 21, 23). A sulfur oxidation route similar to that of *Thiobacillus thiooxidans* has been proposed for *T. ferrooxidans*. The occurrence of sulfur:oxygen oxidoreductase (EC 1.13.11.18), which utilizes molecular oxygen as the electron acceptor, has been reported in *Thiobacillus novellus* (2), *T. ferrooxidans* (13), *T. thiooxidans* (19), and *Thiobacillus thioparus* (20). If an electron acceptor other than molecular oxygen, such as ferric, nitrate, or sulfate ions, is available for sulfur oxidation by *T. ferrooxidans*, a new route for sulfur oxidation other than that previously reported may exist in this organism.

Reduction of the ferric ion by elemental sulfur has been observed in *Sulfolobus acidocaldarius* (1), *T. thiooxidans* (1, 6), and *T. ferrooxidans* (1, 6). In the case of *T. ferrooxidans*, these studies were done mainly in growth experiments, and not only the precise nature of ferric ion reduction, but also the basic fact of whether ferric ion reduction by elemental sulfur proceeds enzymatically or chemically remain to be established. This work showed that the reduction of ferric ion by elemental sulfur was catalyzed by a heat-labile ferric ion-reducing system, and the presence of a new cyclical route for sulfur oxidation in *T. ferrooxidans* is proposed.

MATERIALS AND METHODS

Microorganism, media, and conditions of cultivation. The iron-oxidizing bacterium *T. ferrooxidans* AP19-3 (16) was used throughout this study. The composition of iron-salts medium used throughout this study was as follows: $FeSO_4 \cdot 7H_2O$, 30 g; $(NH_4)_2SO_4$, 3.0 g; KCl, 0.1 g; K_2HPO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $Ca(NO_3)_2$, 0.01 g; deionized water, 1,000 ml; and concentrated H_2SO_4, 0.25 ml (pH 2.5). The method for large-scale production of iron-grown cells, which were used for the measurement of activity of the ferric ion-reducing system, has been previously described (18). Harvested cells were washed three times with sulfuric acid, pH 3.0, and suspended in sulfuric acid, pH 3.0.

Activity of ferric ion-reducing system. The activity of the ferric ion-reducing system that catalyzes the reduction of Fe^{3+} by elemental sulfur was determined by measuring the Fe^{2+} produced in the reaction mixture. The presence of ferrous ion was determined colorimetrically by a modification of the *o*-phenanthroline method as previously described (16). The composition of the reaction mixture used for anaerobic Fe^{3+} reduction by elemental sulfur was as follows: 8 ml of 0.1 M β-alanine–SO_4^{2-} buffer, pH 3.0; elemental sulfur, 200 mg; $FeCl_3 \cdot 6H_2O$, 50 μmol; and washed intact cells of iron-grown *T. ferrooxidans* AP19-3, 4 mg of protein. Total volume was 10.0 ml. A complete reaction mixture was bubbled with nitrogen gas through a narrow glass tube in an ice bath for 10 min. The reduction of Fe^{3+} by elemental sulfur during these treatments was negligible. After the glass tubes were sealed, the reaction mixture was shaken vigorously for 10 s to mix the cells with elemental sulfur, and the reaction was started by shaking the reaction mixture at 30°C. The activity of the ferric ion-reducing system was also measured under aerobic conditions in the presence of 5 mM cyanide, which completely inhibits the reoxidation of Fe^{2+} by inhibiting iron oxidase (see Fig. 2). The composition of the reaction mixture was the same as that used for anaerobic Fe^{3+} reduction, except that sodium cyanide was added to the reaction mixture at 5 mM.

Iron-oxidizing activity. Iron-oxidizing activity was determined by measuring the decrease of Fe^{2+} in the reaction mixture by the *o*-phenanthroline method. The composition of the reaction mixture was as follows: 8 ml of 0.1 M β-alanine–SO_4^{2-} buffer, pH 3.0; washed intact cells of *T.*

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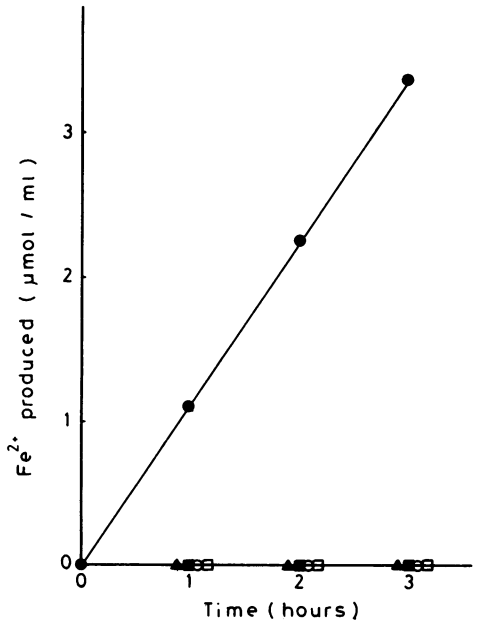

FIG. 1. Reduction of ferric ion with elemental sulfur by washed intact cells of *T. ferrooxidans* AP19-3 under anaerobic conditions. Anaerobic conditions were made by exchanging space in the reaction flask with nitrogen gas. Symbols: ●, Fe^{3+} reduction in a complete reaction mixture; ■, Fe^{3+} reduction in the same reaction mixture as that of a complete system, except that active cells were replaced by cells boiled for 10 min; ▲, Fe^{3+} reduction in a complete reaction mixture lacking active cells; ○, Fe^{3+} reduction in a complete reaction mixture lacking elemental sulfur; □, Fe^{3+} reduction in a complete reaction mixture lacking Fe^{3+}.

ferrooxidans AP19-3, 4 mg of protein; and $FeSO_4 \cdot 7H_2O$, 0.5 mmol. Total volume was 10.0 ml. The reaction was started by shaking the reaction mixture at 30°C.

Sulfur-oxidizing activity. Sulfur-oxidizing activity was determined manometrically by measuring the oxygen uptake caused by the oxidation of elemental sulfur. The composition of the reaction mixture was as follows: 2 ml of 0.05 M β-alanine–HCl buffer, pH 3.0; intact cells which had been washed three times with 0.05 M β-alanine–HCl buffer, pH 3.0, to wash sulfate from the cells, 4 mg of protein; and elemental sulfur, 30 mg. Total volume was 3.2 ml. The reaction was performed at 30°C. Sulfur-oxidizing activity was also determined by measuring the increase of sulfate production in the reaction mixture. The composition of the reaction mixture was as follows: 8 ml of 0.05 M β-alanine–HCl buffer, pH 3.0; elemental sulfur, 200 mg; and intact cells which had been washed three times with 0.05 M β-alanine–HCl buffer, pH 3.0, to wash sulfate from the cells, 4 mg of protein. Total volume was 10.0 ml. After shaking the reaction mixture vigorously for 10 s to mix the cells with elemental sulfur, the reaction was started by shaking the reaction mixture at 30°C. A sample of the reaction mixture was withdrawn after some time, and the concentration of sulfate was determined by the method described below.

Analysis. The amount of sulfate ion (SO_4^{2-}) was determined spectrophotometrically. A sample of the reaction mixture (1.5 ml) was centrifuged at 10,000 × *g* for 5 min to discard cells and solid elemental sulfur. Barium chloride solution (1 ml) and 0.1 N HCl (1 ml) were added to 1 ml of the supernatant solution obtained by centrifugation. Total volume was 3.0 ml. A resulting white turbidity due to barium sulfate was measured by a Hitach 101 spectrophotometer at 550 nm. A good linearity was obtained between sulfate concentration (0.1 to ~1.0 μmol of SO_4^{2-}) and turbidity.

The amount of sulfite ion (SO_3^{2-}) was determined spectrophotometrically. A sample of the reaction mixture (1.5 ml) was centrifuged at 10,000 × *g* for 5 min to discard cells and solid elemental sulfur. Zinc acetate solution (2%; 1 ml), fuchsin-sulfuric acid solution (0.2 ml), and 1.9% formaldehyde solution (0.2 ml) were added to 1 ml of the supernatant solution obtained by centrifugation. Total volume was 3.0 ml. A good linearity was obtained between sulfite concentration (0.01 to ~0.1 μmol of SO_3^{2-}) and the developed purple color that was measured by a Hitachi 101 spectrophotometer at 570 nm. The protein content was determined by the biuret method (9), with crystalline bovine serum albumin as the reference protein.

RESULTS

Properties of the ferric ion-reducing system in *T. ferrooxidans*. The activity of the ferric ion-reducing system

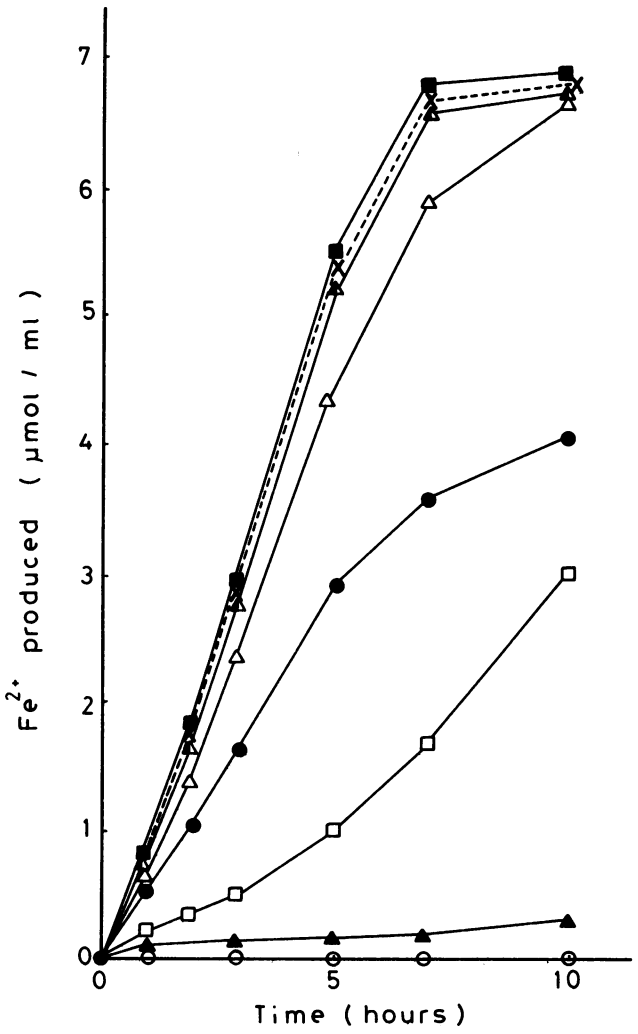

FIG. 2. Reduction of ferric ion with elemental sulfur by washed intact cells of *T. ferrooxidans* AP19-3 under aerobic conditions. Symbols indicate the concentration of sodium cyanide added to a complete reaction mixture: ○, 0; ▲, 1 μM; □, 10 μM; △, 0.1 mM; ■, 5 mM; ▲, 10 mM; ●, 50 mM. As a control, the reduction of Fe^{3+} under anaerobic conditions was performed with a complete reaction mixture lacking cyanide (×).

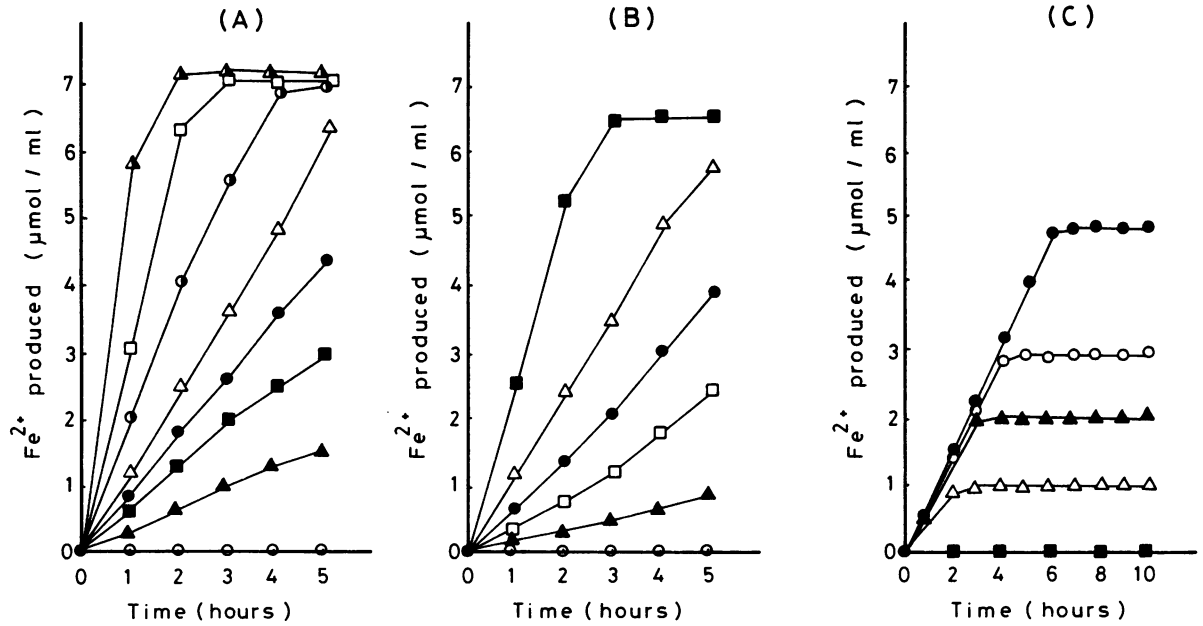

FIG. 3. Effects of the concentrations of cells, elemental sulfur, and ferric ion on the activity of the ferric ion-reducing system. The reaction was performed under aerobic conditions in the presence of 5 mM cyanide. (A) Effect of cell concentration on the activity of Fe^{3+} reduction. Symbols: ○, no protein; ▲, 0.5 mg of protein; ■, 1.0 mg of protein; ●, 2.0 mg of protein; △, 4 mg of protein; ◑, 10 mg of protein; □, 20 mg of protein; ▲, 40 mg of protein. (B) Effect of elemental sulfur concentration on the activity of Fe^{3+} reduction. Symbols: ○, no sulfur; ▲, 10 mg of sulfur; □, 50 mg of sulfur; ●, 100 mg of sulfur; △, 200 mg of sulfur; ■, 500 mg of sulfur. (C) Effect of Fe^{3+} concentration on the activity of Fe^{3+} reduction; ■, no Fe^{3+}; △, 10 μmol of Fe^{3+}; ▲, 20 μmol of Fe^{3+}; ○, 30 μmol of Fe^{3+}; ●, 50 μmol of Fe^{3+}.

which catalyzes the reduction of ferric ion with elemental sulfur was studied with washed intact cells of iron-grown *T. ferrooxidans* AP19-3. The activity of intact cells was stable for preservation at 4°C for about 5 weeks, but very unstable to mechanical treatment such as freezing or sonication. Since no attempts to obtain an active cell extract of the ferric ion-reducing system from the strain have done well, all experiments presented in this text were performed with washed intact cells.

Under anaerobic condition, washed intact cells reduced Fe^{3+} with elemental sulfur, but cells boiled for 10 min could not reduce Fe^{3+} (Fig. 1). No Fe^{2+} production was observed in those reaction mixtures lacking elemental sulfur, active cells, or Fe^{3+}. Brock and Gustafson showed that Fe^{3+} in an anaerobic growth culture of *T. ferrooxidans* was reduced with elemental sulfur (1). *T. thiooxidans* reduced Fe^{3+}, but the rate and amount of Fe^{3+} reduction were very much lower than those of *T. ferrooxidans* (data not shown). Under aerobic condition, no Fe^{2+} production was observed (Fig. 2). However, if sodium cyanide was added to the reaction mixture, Fe^{2+} production was observed under aerobic conditions. The amount of Fe^{3+} reduction by elemental sulfur was proportional to the concentration of cyanide added to the reaction mixture. These results suggest that cyanide inhibited the iron oxidase of the cell, but did not inhibit the ferric ion-reducing system. We previously showed that cyanide completely inhibited iron oxidase at 0.1 mM, cytochrome *c* oxidase at 1 mM (4, 18), and the energy-dependent uptake of glutathione at 1 mM (17). The amount of Fe^{2+} production in the reaction mixture supplemented with 5 mM cyanide was comparable to that obtained under anaerobic conditions lacking cyanide. So the properties of the ferric ion-reducing system were determined by using the reaction mixture supplemented with 5 mM cyanide and under aerobic conditions unless indicated otherwise.

When we incubated intact cells for 10 min at 55°C, the activity of the ferric ion-reducing system decreased about 65%, and activity was completely destroyed by a 10-min incubation at 60°C. The pH optimum for the ferric ion-reducing system was between 2.0 and 3.8, and no Fe^{3+} reduction was observed below pH 1.4 on above pH 4.8. The rate of Fe^{3+} reduction was proportional to the concentration of cells, elemental sulfur, and Fe^{3+} in the reaction mixture (Fig. 3). Fe^{3+} reduction continued until all the Fe^{3+} in the reaction mixture was reduced to Fe^{2+}.

Stoichiometry of anaerobic sulfur oxidation by ferric ion. The stoichiometry of anaerobic sulfur oxidation by Fe^{3+} was investigated with washed intact cells of *T. ferrooxidans* AP19-3. The amounts of Fe^{2+}, sulfate, and sulfite in the reaction mixture were determined throughout the reaction (Fig. 4). To determine the concentration of sulfate, cells were washed three times with 0.05 M β-alanine–HCl buffer, pH 3.0, and this buffer was also used for the reaction. The amount of sulfate that leaked from the cells into the reaction mixture was negligible throughout the reaction. No sulfate could be detected in the reaction mixture lacking elemental sulfur or Fe^{3+}. An increase in the amount of Fe^{2+} produced by sulfur oxidation under anaerobic conditions accompanied a concomitant increase in sulfate production. The molar ratio of sulfate to Fe^{2+} was 0.95 to ~1.15:6.0. These results suggest that 1 mol of sulfate was produced when 6 mol of Fe^{3+} was reduced by elemental sulfur.

Besides sulfate, a small amount of sulfite was also detected in the reaction mixture. Cells boiled for 10 min could not produce sulfite. After 30 min of the reaction, the increase in sulfite production was stopped, and then the sulfite level gradually decreased despite the increase in both Fe^{2+} and sulfate levels. The results suggests that sulfite is an intermediate compound in elemental sulfur oxidation by Fe^{3+}. Since sulfite is a well-known, potent reducing agent, its disappear-

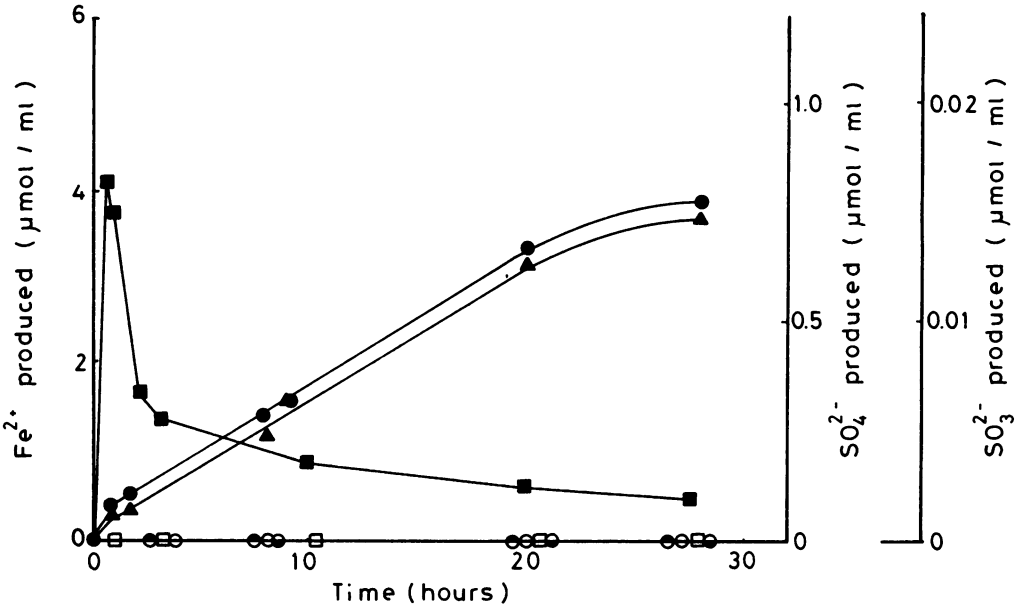

FIG. 4. Production of ferrous, sulfate, and sulfite ions in anaerobic oxidation of elemental sulfur with ferric ion by washed intact cells of *T. ferrooxidans* AP19-3. Symbols: ●, Fe^{2+} production in a complete reaction mixture; ▲, SO_4^{2-} production in a complete reaction mixture; ○, SO_4^{2-} production in a complete reaction mixture lacking cells; ◒, SO_4^{2-} production in a complete reaction mixture lacking elemental sulfur; ◒, SO_4^{2-} production in a complete reaction mixture lacking Fe^{3+}; ■, SO_3^{2-} production in a complete reaction mixture; □, SO_3^{2-} production in a complete reaction mixture except that active cells were replaced by cells boiled for 10 min.

ance from the reaction mixture may be due to its chemical oxidation by Fe^{3+}.

Stoichiometry of sulfur oxidation by molecular oxygen. The stoichiometry of sulfur oxidation by molecular oxygen was investigated with washed intact cells of *T. ferrooxidans* AP19-3. The washed intact cells and buffer solution used in this experiment were the same those described in Fig. 4, except that Fe^{3+} was omitted and the reaction was carried out in air. The amounts of Fe^{2+}, sulfate, and sulfite in the reaction mixture were determined throughout the reaction (data not shown). It was found that the rate of sulfate production in the reaction mixture was much higher than the rate of production under anaerobic conditions and that Fe^{2+} was not produced. The amount of sulfite produced by sulfur oxidation under aerobic conditions was much lower than that produced under unaerobic conditions. Under aerobic conditions, the strain could oxidize sulfite added to the reaction mixture, but it could not oxidize sulfite in the presence of 0.5 mM sodium cyanide (Fig. 5), suggesting that iron oxidase in the strain is involved in aerobic sulfite oxidation.

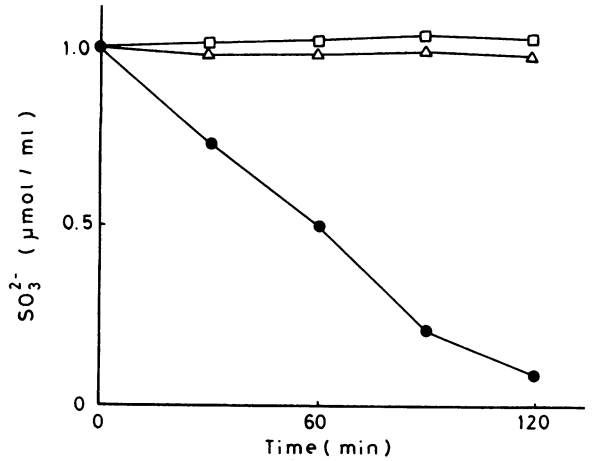

FIG. 5. Oxidation of sulfite by *T. ferrooxidans* AP19-3. The reaction was performed under aerobic conditions with intact cells washed with 0.05 M β-alanine–HCl buffer, pH 3.0. The composition of a complete reaction mixture was as follows: 8 ml of 0.05 M β-alanine–HCl buffer, pH 3.0; washed intact cells, 4 mg of protein; and Na_2SO_3, 10 μmol. Total volume was 10.0 ml. Symbols: ●, SO_3^{2-} oxidation in a complete reaction mixture; △, SO_3^{2-} oxidation in a complete reaction mixture supplemented with 0.5 mM sodium cyanide; □, SO_3^{2-} oxidation in a complete reaction mixture except that intact cells were replaced by intact cells boiled for 10 min.

The relationship between oxygen consumption and sulfate production was investigated with washed intact cells. Oxygen uptake caused by the oxidation of elemental sulfur was determined manometrically, and the amount of sulfate produced was analyzed. It was found that 1 μmol of sulfate was produced when 1.5 μmol of molecular oxygen was consumed (data not shown).

Involvement of the ferric ion-reducing system in sulfur oxidation of *T. ferrooxidans* AP19-3. The washed intact cells (4 mg of protein) of *T. ferrooxidans* AP19-3 were incubated in 10 ml of 0.5% phenol or in 10 ml of water for 10 min at 30°C. After the treatment, the cells were washed three times with water and with 0.05 M β-alanine–HCl buffer, pH 3.0. The activities of iron oxidase, the ferric ion-reducing system, and aerobic sulfur oxidation were determined by the methods described above. The activities of both the ferric ion-reducing system and aerobic sulfur oxidation in 0.5% phenol-treated cells were completely destroyed (Fig. 6). In contrast, iron oxidase in 0.5% phenol-treated cells remained still active. The results suggest that a short treatment of the strain with 0.5% phenol selectively destroyed the ferric ion-reducing system of the cell. The cells treated with 2.0% phenol for 10 min at 30°C completely lost all activities, including iron oxidase activity.

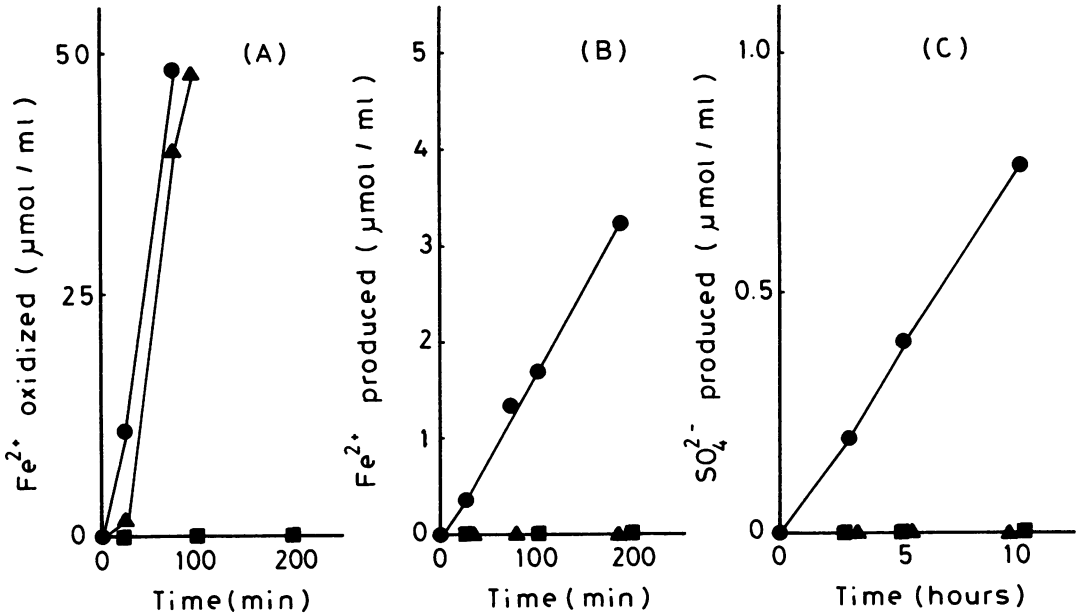

FIG. 6. Activities of iron oxidase (A), the ferric ion-reducing system (B), and aerobic sulfur oxidation (C) in phenol-treated or untreated *T. ferrooxidans* AP19-3. Symbols: ●, intact cells untreated with phenol; ▲, 0.5% phenol-treated cells; ■, 2.0% phenol-treated cells.

The intact cells treated with 5 mM sodium sulfite for 20 min at 30°C and washed three times with water and with 0.05 M β-alanine–HCl buffer, pH 3.0, showed results similar to those obtained with 0.5% phenol-treated cells (data not shown). The concomitant destruction of the activity of aerobic sulfur oxidation by molecular oxygen in both 0.5% phenol- and 5 mM sulfite-treated cells strongly suggests that the ferric ion-reducing system plays an important role in sulfur oxidation by the strain. The compounds that strongly inhibit the activities of both the ferric ion-reducing system and iron oxidase, such as mercuric ion and sodium dodecyl sulfate, or cyanide, that specifically inhibits iron oxidase, markedly inhibited the activity of sulfur oxidation by molecular oxygen.

DISCUSSION

The properties of the ferric ion-reducing system that catalyzes the reduction of Fe^{3+} by elemental sulfur were investigated with intact cells of *T. ferrooxidans* AP19-3. The ferric ion-reducing system, which appears to be composed of a series of enzymes, was heat labile and unstable when subjected to mechanical treatment. This system is distinguished from that of sulfur:oxygen oxidoreductase, which is involved in sulfur oxidation in *T. novellus*, *T. ferrooxidans*, *T. thiooxidans*, and *T. thioparus* (2, 13, 19, 20), by the following points: (i) as a specific electron acceptor for elemental sulfur, the ferric ion-reducing system utilizes Fe^{3+}, but the sulfur:oxygen oxidoreductase system utilizes molecular oxygen; (ii) the ferric ion-reducing system is not inhibited by a higher concentration of Fe^{2+} or Fe^{3+}, but the sulfur:oxygen oxidoreductase system is strongly inhibited by both Fe^{2+} and Fe^{3+} at 1 mM; (iii) cyanide has no effect on the ferric ion-reducing system, but it strongly inhibits the sulfur:oxygen oxidoreductase system.

Ottow and von Klopotek observed the capacity for Fe^{3+} reduction in some fungi and bacteria that have nitrate reductase and concluded that Fe^{3+} reduction was catalyzed by nitrate reductase (10, 11). However, the ferric ion-reducing system should be distinguished from the nitrate reductase system, because no ammonium or nitrite ions were detected in the reaction mixture in which Fe^{3+} was replaced by nitrate. Strain AP19-3 could utilize nitrate as a sole source of nitrogen under aerobic conditions, but could not grow anaerobically on sulfur-salts medium with nitrate as a sole source of electron acceptor.

The ferric ion-reducing system appears to play an important role in sulfur oxidation in this strain, because a selective loss from the strain of activity of the system accompanied a loss of the ability to oxidize elemental sulfur by molecular oxygen. A specific role for the enzyme system in sulfur oxidation in the strain was also supported by the fact that strain AP19-3 shows a much higher activity for this enzyme system than do intact cells of *T. thiooxidans*. By using a cell-free system, Kino and Usami reported that Fe^{3+} reduction in *T. thiooxidans* seems to be a nonenzymatic process (6).

From the results shown in Fig. 4, the following equations were proposed for anaerobic sulfur oxidation by Fe^{3+}. In the first step of the reaction, 4 mol of Fe^{3+} is reduced by 1 mol of elemental sulfur to give 4 mol of Fe^{2+} and 1 mol of sulfite.

$$S^0 + 4FeCl_3 + 3H_2O \rightarrow H_2SO_3 + 4FeCl_2 + 4HCl \quad (1)$$

This process may be catalyzed by the ferric ion-reducing system of the cell, which appears to be composed of a series of enzymes. In the second step, 1 mol of sulfite produced as shown in equation 1 chemically reduces 2 mol of Fe^{3+} to give 2 mol of Fe^{2+} and 1 mol of sulfate.

$$H_2SO_3 + 2FeCl_3 + H_2O \rightarrow H_2SO_4 + 2FeCl_2 + 2HCl \quad (2)$$

The total reaction of anaerobic sulfur oxidation by Fe^{3+} is shown by a summary of equations 1 and 2, so we have the following:

$$S^0 + 6FeCl_3 + 4H_2O \rightarrow H_2SO_4 + 6FeCl_2 + 6HCl \quad (3)$$

Equation 3 suggests that 1 mol of sulfate and 6 mol of Fe^{2+} should be produced by the anaerobic oxidation of 1 mol of elemental sulfur by Fe^{3+}. The fact that no Fe^{2+} was ob-

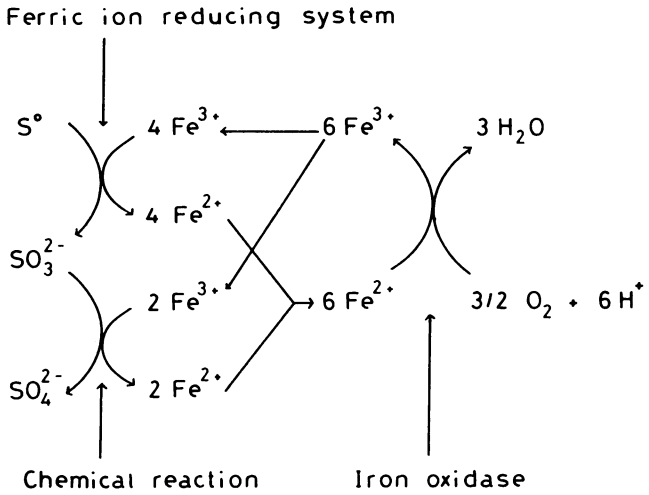

FIG. 7. New route proposed for sulfur oxidation in *T. ferrooxidans* AP19-3.

served under aerobic conditions strongly suggests that the 6 mol of Fe^{2+} produced by the reaction shown in equation 3 is immediately oxidized as shown in equation 4, which is the same equation as that for iron oxidation in *T. ferrooxidans*, as follows:

$$6FeCl_2 + 6HCl + 3/2O_2 \rightarrow 6FeCl_3 + 3H_2O \quad (4)$$

If the hypothesis is true that Fe^{2+} produced by anaerobic sulfur oxidation is oxidized by the iron oxidase of the cell, the total reaction for aerobic oxidation of elemental sulfur can be written as shown in equation 5, which is a summary of equations 3 and 4.

$$S^0 + 3/2O_2 + H_2O \rightarrow H_2SO_4 \quad (5)$$

According to equation 5, 1 mol of sulfate will be produced when 1.5 mol of molecular oxygen is consumed, and this fact was ascertained in this study. In this way, in the case of *T. ferrooxidans* AP19-3, aerobic sulfur oxidation can be explained by the following three-step reaction: (i) the ferric ion-reducing system reduces Fe^{3+} with elemental sulfur to give Fe^{2+} and sulfite; (ii) the sulfite is chemically oxidized by Fe^{3+} to give Fe^{2+} and sulfate; and (iii) the resulting Fe^{2+} is oxidized by iron oxidase to complete a cyclical process (Fig. 7).

Besides using a conventional sulfur oxidation route, the strain may oxidize some part of elemental sulfur in the medium by using this new sulfur oxidation route. The problem of what percentage of elemental sulfur is oxidized by the new route is not clear and is now under investigation. It appears possible that the strain can oxidize elemental sulfur by the ferric ion-reducing system under those undesirable conditions in which the concentration of oxygen available to the cells is lower than that of optimal growth conditions or when some compounds that inhibit the iron oxidase of the cell are present in their environment.

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