Comparative Bioenergetics of Sulfate Reduction in
*Desulfovibrio* and *Desulfotomaculum* spp.

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Extracts of *Desulfotomaculum nigrificans*, *Desulfotomaculum orientis*, and *Desulfotomaculum ruminis* exhibit low levels of inorganic pyrophosphatase but were found to have high levels of pyrophosphate-acetate phosphotransferase. Conversely, extracts of *Desulfovibrio gigas*, *Desulfovibrio vulgaris*, and *Desulfovibrio desulfuricans* Norway 4 were shown to have high levels of inorganic pyrophosphatase but negligible amounts of pyrophosphate-acetate phosphotransferase. Both enzymes are reductant activated and appear to have an analogous function in removing pyrophosphate formed during the activation of sulfate. Conservation of the bond energy of pyrophosphate in *Desulfotomaculum* eliminates the necessity for invoking electron-transfer-coupled phosphorylation to account for the growth of these bacteria on lactate plus sulfate. Relative growth yields of *Desulfovibrio vulgaris* and *Desulfotomaculum orientis* on lactate plus sulfate indicate that the latter does not carry out significant electron-transfer-coupled phosphorylation in this mode of growth.

The bioenergetics of sulfate reduction in the two established genera of the sulfate-reducing bacteria, *Desulfovibrio* (32) and *Desulfotomaculum* (12), have been generally believed to be identical and to involve electron-transfer-coupled phosphorylation. Microorganisms in the genus *Desulfovibrio* are characterized as anaerobic gram-negative non-sporeforming vibrios which can utilize sulfate as a terminal electron acceptor with hydrogen and during growth on a limited number of organic substrates. They contain both b- and c-type cytochromes and, with one exception (24), desulfoviridin (bisulfite reductase), which is of taxonomic importance for the genus because of the intense red fluorescence produced in alkali (30). Microorganisms in the genus *Desulfotomaculum* are characterized as anaerobic sporeforming gram-negative rods which can utilize sulfate as a terminal electron acceptor during growth on a limited number of organic substrates. They possess only a b-type cytochrome and lack desulfoviridin. Instead, they contain an analogous bisulfite reductase (38) with a major absorption at 582 nm which does not fluoresce in alkali. Immunologically there is little cross-reaction between antibodies and crude proteins of the two genera, suggesting that the two groups of bacteria are quite distinct (31).

The genus *Desulfovibrio* has been most extensively studied as regards the physiology and biochemistry of sulfate reduction, and the conclusions have been largely transferred to *Desulfotomaculum*. Biochemically, the pathway of sulfate reduction to sulfite appears to be quite similar in both genera and is outlined in equations 1 to 3,

\[
\begin{align*}
\text{ATP sulfurylase: } & \text{ATP} + \text{SO}_4^{2-} \rightarrow \text{APS} + \text{PP}^1 \\
\text{PPase: } & \text{PP}^1 + \text{H}_2\text{O} \rightarrow 2\text{P}^1 \\
\text{APS reductase: } & \text{APS} + 2e^{-} \rightarrow \text{SO}_4^{2-} + \text{AMP}
\end{align*}
\]

where APS is adenylylsulfate and PPase is inorganic pyrophosphatase. The equilibrium of the reaction catalyzed by ATP sulfurylase (equation 1) lies in the direction of ATP and SO$_4^{2-}$, and it is thought that PPase (equation 2) is required to "pull" the reaction in the direction of APS formation. ATP sulfurylase (3) has been partially purified from *Desulfotomaculum nigrificans* (formerly *Clostridium nigrificans*) and *Desulfovibrio vulgaris* (formerly *Desulfovibrio desulfuricans*). APS reductase has been purified and studied from *Desulfovibrio vulgaris* (8) and is found in all sulfate-reducing bacteria (37). PPase, which is reductant activated (40), has been partially purified from *Desulfovibrio vulgaris* (4); however, the presence of PPase has not been reported in *Desulfotomaculum*.

*Desulfovibrio* was the first non-photosynthetic anaerobe in which a c-type cytochrome (cytochrome c$_3$; $M_r = 13,000$) was demonstrated (29), and the cytochrome is exceptional in the biological world in that the molecule contains four independent heme groups (18). The cytochrome has been considered as somehow unique to respiratory sulfate reduction, but its exact physiological role(s) in this process has remained
elusive. Recently Wood (45) has questioned whether there is any relationship between this cytochrome and the phenomenon of respiratory sulfate reduction. These bacteria were the first anaerobes in which electron-transfer-coupled phosphorylation was demonstrated (26, 28), and because of the requirement for the hydrolysis of two phosphate groups of ATP for the formation of APS, it is necessary that the bacteria carry out electron-transfer phosphorylation even when growing on organic substrates such as lactate and ethanol (27). These bioenergetic considerations require electron-transfer phosphorylation for the reduction of sulfate with H₂, and growth yield studies with a new strain of Desulfovibrio vulgaris capable of growth on H₂, CO₂, SO₄²⁻, and acetate indicate that three molecules of ATP are generated during the reduction of sulfate to sulfide with hydrogen, but that ATP formation is not coupled to the reduction of APS to sulfide (5). These data also indicate that PPase is involved in the mechanism of formation of APS by growing cells of Desulfovibrio.

The metabolism of molecular hydrogen has figured centrally in the development of our present concepts concerning the bioenergetics and physiology of respiratory sulfate reduction, and hydrogenase has been extensively studied. All described species of Desulfovibrio contain hydrogenase in high specific activity (35), which is either soluble and largely localized in the periplasmic space (7) or bound to the cytoplasmic membrane (46). The enzyme is a non-heme iron protein containing three [Fe₃S₄] clusters per molecular weight of 50,000 (39) and requires cytochrome c₅ (Mₚ = 13,000) for activity with naturally occurring electron acceptors (6). In contrast to Desulfovibrio, the hydrogen metabolism of Desulfitomaculum has been less well studied and appears to be quite different from that of Desulfovibrio. Desulfitomaculum orientis has been demonstrated to lack hydrogenase (1) and thus does not utilize or produce hydrogen. A number of strains of Desulfitomaculum nigrificans do not contain hydrogenase (31), but in those that do contain the enzyme, it is membrane bound and of low specific activity (2). There is no report of the reduction of sulfate with hydrogen by Desulfitomaculum nigrificans. Desulfitomaculum ruminis contains a membrane-bound hydrogenase of low specific activity (11) which is localized on the inner surface of the cytoplasmic membrane (unpublished data). Desulfitomaculum ruminis has been reported to reduce sulfate with hydrogen; however, the cells require the presence of 2% yeast extract (13). These scattered observations imply that the role of hydrogen in the metabolism of Desulfitomaculum is quite limited and different from that of Desulfovibrio.

In this communication, we report the virtual absence of PPase in extracts of three species of Desulfitomaculum and its apparent replacement by pyrophosphatase:acetate phosphotransferase (PP::acetate kinase) (34). Evidence is presented to indicate that, because of the conservation of the bond energy of the pyrophosphate produced during the formation of APS, the sporforming sulfate-reducing bacteria do not carry out electron-transfer-coupled phosphorylation under usual growth conditions.

MATERIALS AND METHODS

Organisms. Desulfitomaculum ruminis 42 and Desulfitomaculum nigrificans ATCC 19968 were kindly supplied by J. M. Akagi, and Desulfitomaculum orientis ATCC 19365 was purchased from the American Type Culture Collection. Desulfovibrio gigas NCIB was originally obtained from Jean LeGall, Desulfovibrio vulgaris NCIB 8303 and Desulfovibrio desulfuricans Norway 4 (NCIB 8310) were purchased from the National Collection of Industrial Bacteria. All cultures were routinely transferred by means of the Hungate technique (16) on a modified LeGall medium (20) and incubated at 37°C, except for Desulfitomaculum nigrificans, which was incubated at 55°C.

Preparation of whole cells. The microorganisms were grown in carboys as previously described (17) and harvested in the late log phase of growth. FeS was removed by filtering the medium through several layers of cotton gauze, and the cells were collected by centrifugation in a Sorvall RC-2B centrifuge at 10,000 × g for 10 min. The cells were washed three to four times with distilled water before use.

Preparation of extracts. Cell-free extracts were prepared by suspending the wet cell paste in 0.1 M Tris buffer (pH 8.0; 1:2, wt/vol) and passing the suspension through an Amino French pressure cell at 8,000 lb/in². The preparation was then centrifuged at 10,000 × g for 30 min at 4°C, the pellets were discarded, and the supernatant fluid was recentrifuged at 100,000 × g for 90 min in a Beckman ultracentrifuge, model L. Streptomycin sulfate (0.5 mg/mg of protein) was next added to the supernatant, and, after centrifugation, the extract was dialyzed overnight against 0.01 M Tris buffer (pH 8).

Assays. ATP sulfurylase was determined with MoO₄²⁻ as described by Wilson and Bandurski (43), and PPase was determined by the method of Akagi and Campbell (3). PP::acetate kinase was assayed by using the conditions of Reeves and Guthrie (34), except that acetyl phosphate (22) produced from acetate plus pyrophosphate was determined. Sulfide was measured by the method of Siegel (36), and protein was measured by the biuret method (21). Lactate and acetate were measured with a Varian Aerograph 2700 gas chromatograph equipped with a hydrogen flame ionization detector.

Fractionation of PPase and PP::acetate kinase.
Crude extracts of Desulfitomaculum ruminis and Desulfovibrio vulgaris were fractionated with solid ammonium sulfate (enzyme grade) from 0 to 30 and 30 to 50% saturation. The 50% (NH₄)₂SO₄ supernatant was dialyzed overnight against 10⁻² M Tris buffer (pH 8.0), and the protein was then applied to a DEAE-52 column (4.0 by 35 cm) equilibrated with 10⁻² M Tris buffer (pH 8.0). The protein was eluted by means of a linear Tris gradient (pH 8.0) from 0.01 to 0.4 M, and 10-ml fractions were collected and assayed for PPase, PP-acetate kinase, and protein.

**RESULTS**

**Whole cell studies.** We were unable to detect the reduction of sulfate with H₂ by using intact cells of either Desulfitomaculum nigrificans or Desulfitomaculum ruminis; however, it was possible to observe the reduction of sulfite and thiosulfate with H₂. The reduction of sulfate was also obtained with substrate amounts of pyruvate, which serves both as an electron donor and energy source, but it was not possible to stimulate the reduction of sulfate with H₂ by catalytic amounts of sulfite or thiosulfate (26). This major difference in capability between Desulfitomaculum and Desulfovibrio did not appear to originate from the lysis of cells, low levels of hydrogenase, inactivation of enzymes, or dilution of cofactors, and suggested that there were fundamental differences in the bioenergetics of sulfate reduction between Desulfovibrio and Desulfitomaculum.

**Effect of PPase and acetate on ATP sulfurylase.** ATP sulfurylase, determined in the presence of molybdate ion by the assay of orthophosphate, requires the presence of PPase (43). As shown in Table 1, extracts of Desulfitomaculum nigrificans, Desulfitomaculum orientis, and Desulfitomaculum ruminis exhibited low levels of ATP sulfurylase activity, whereas extracts of Desulfovibrio vulgaris, Desulfovibrio gigas, and Desulfovibrio desulfuricans showed levels of activity comparable to those previously reported (3, 25). The addition of PPase to the three extracts of Desulfitomaculum stimulated ATP sulfurylase from 3- to 39-fold, whereas ATP sulfurylase in the three extracts of Desulfovibrio was at most stimulated by 50%. These preliminary observations suggested that the pyrophosphate produced concomitantly with the formation of APS in Desulfitomaculum might be removed by a mechanism different from that in Desulfovibrio. Although a number of phosphotransferase reactions involving pyrophosphate have been reported (44), a likely candidate for the removal of pyrophosphate in these organisms appeared to be the PPi:acetate kinase from Entamoeba histolytica described by Reeves and Guthrie (34). The reaction catalyzed by this enzyme is shown in equation 4:

\[ \text{Acetate} + \text{PPi} = \text{Acetyl phosphate} + \text{P}_i \] (4)

Reeves and Guthrie reported the reaction to be reversible, but the rate in the direction of acetyl phosphate formation is only 2% of the rate in the direction of PPi formation. ATP did not replace PPi as phosphoryl donor, and none of the common nucleoside diphosphates replaced orthophosphate as phosphoryl acceptor. The effect of acetate on ATP sulfurylase activities in crude extracts of Desulfovibrio vulgaris and the three species of Desulfitomaculum is shown in Table 2. Acetate stimulated ATP sulfurylase from 7- to 50-fold in the extracts of Desulfitomaculum.

**TABLE 1. Effect of PPase on ATP sulfurylase activity in crude extracts of desulfovibrio and desulfitomaculum**

<table>
<thead>
<tr>
<th>Organism</th>
<th>ATP sulfurylase activity (µmol of P₃ min⁻¹ mg⁻¹) (10⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>− PPase</td>
</tr>
<tr>
<td>Desulfitomaculum orientis</td>
<td>14</td>
</tr>
<tr>
<td>Desulfitomaculum ruminis</td>
<td>9</td>
</tr>
<tr>
<td>Desulfitomaculum nigrificans</td>
<td>0</td>
</tr>
<tr>
<td>Desulfovibrio vulgaris</td>
<td>58</td>
</tr>
<tr>
<td>Desulfovibrio gigas</td>
<td>119</td>
</tr>
<tr>
<td>Desulfovibrio desulfuricans</td>
<td>58</td>
</tr>
<tr>
<td>Norway 4</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 2. Effect of acetate on ATP sulfurylase activity in crude extracts of desulfovibrio and desulfitomaculum**

<table>
<thead>
<tr>
<th>Organism</th>
<th>ATP sulfurylase activity (µmol of P₃ min⁻¹ mg⁻¹) (10⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>− Acetate</td>
</tr>
<tr>
<td>Desulfitomaculum ruminis</td>
<td>9</td>
</tr>
<tr>
<td>Desulfitomaculum nigrificans</td>
<td>0</td>
</tr>
<tr>
<td>Desulfovibrio vulgaris</td>
<td>59</td>
</tr>
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</table>
maculum and was dependent on the presence of MoO$_4^{2-}$. No stimulation of activity was observed in the extract of Desulfovibrio vulgaris.

Specific activities of PPase and PP$_i$:acetate kinase. The specific activities of PPase and PP$_i$:acetate kinase in extracts of Desulfovibrio and Desulfotomaculum are indicated in Table 3. The values for PPase have been divided by 2 so that the values for both enzymatic activities will reflect the hydrolysis of the pyrophosphate bond. The specific activity of PPase varied from 16 to 21 in extracts of Desulfotomaculum and from 140 to 275 in extracts of Desulfovibrio. Conversely, the specific activity of PP$_i$:acetate kinase varied from 81 to 122 in extracts of Desulfotomaculum and from 5 to 10 in extracts of Desulfovibrio. These data clearly indicate that a major difference exists between Desulfovibrio and Desulfotomaculum in the metabolism of pyrophosphate, which is significant for the bioenergetics of sulfate reduction in these strains. The specific activity of the PP$_i$:acetate in the three extracts of Desulfotomaculum, although somewhat lower than that of PPase in Desulfovibrio, was consistent with the involvement of this enzyme in the energy metabolism of Desulfotomaculum.

Chromatography of the crude extract of Desulfovibrio vulgaris. To determine whether the low level of PP$_i$:acetate kinase activity in crude extracts of Desulfovibrio was due to the actual presence of the enzyme or to nonspecific reactions, an extract of Desulfovibrio vulgaris was chromatographed on DEAE-52, and the distribution of both PPase and PP$_i$:acetate kinase was determined in the eluted protein fractions. A single peak of PPase activity was obtained (Fig. 1), but we were unable to detect phosphotransferase in any of the protein fractions. The PPase activity was stimulated by dithionite as reported by Ware and Postgate (40), even though the whole cells were not shaken with O$_2$ before preparation of the extract. These data establish that PP$_i$:acetate kinase is absent in cells of Desulfovibrio vulgaris grown on lactate plus sulfate. The low activity observed in crude extracts is in all probability due to nonspecific reactions.

Chromatography of the crude extract of Desulfotomaculum ruminis. To determine whether the low level of PPase activity observed in crude extracts of Desulfotomaculum was due to the actual presence of PPase or to nonspecific reactions, an extract of Desulfotomaculum ruminis was chromatographed on DEAE-52, and the distribution of both PPase and PP$_i$:acetate kinase was determined in the eluted protein fractions. The PP$_i$:acetate kinase was eluted as a single peak of activity (Fig. 2), and activity was stimulated by dithionite just as in the case of the PPase activity of Desulfovibrio. This observation suggests that the PPase and PP$_i$:acetate have analogous functions in Desulfovibrio and Desulfotomaculum and that the regulatory function proposed by Ware and Postgate (40) for the reductant-activated PPase also applies to the PP$_i$:acetate kinase. A small peak of PPase activity was detected, but it was neither coincidental with the PP$_i$:acetate kinase activity nor stimulated by dithionite. It is not surprising that the crude extract of Desulfotomaculum ruminis shows a trace of PPase activity, but whether the activity is specific or nonspecific has not yet been further investigated.

Comparative growth yields of Desulfovibrio and Desulfotomaculum. The low activity of PPase and the unique presence of PP$_i$:acetate kinase which is reductant activated in extracts of Desulfotomaculum suggest that these organisms are able to conserve the energy of the pyrophosphate bond during sulfate reduction by the following series of reactions:

\[
\text{ATP} + \text{SO}_4^{2-} \rightleftharpoons \text{APS} + \text{PP}_i
\]

\[
\text{PP}_i + \text{acetate} \rightleftharpoons \text{Acetyl phosphate} + \text{Pi}
\]

\[
\text{Acetyl phosphate} + \text{ADP} \rightleftharpoons \text{acetate} + \text{ATP}
\]

Acetate kinase (equation 5) is present in all extracts of Desulfotomaculum used in this investigation as well as in Desulfovibrio desulfuricans (9). By means of this mechanism the energy requirement for sulfate reduction is one ATP per sulfate reduced in Desulfotomaculum compared to two ATPs in Desulfovibrio. In terms of growth on lactate plus sulfate, Desulfotomaculum should be capable of producing a net of one ATP per sulfate and not have an obligatory requirement for electron-transfer.

**Table 3. Specific activities of PPase and PP$_i$:acetate-kinase in extracts of sulfate-reducing bacteria.**

<table>
<thead>
<tr>
<th>Organism</th>
<th>PPase activity (10$^{-3}$)*</th>
<th>PP$_i$: acetate kinase activity (10$^{-3}$)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desulfotomaculum orientis</td>
<td>21</td>
<td>82</td>
</tr>
<tr>
<td>Desulfotomaculum ruminis</td>
<td>20</td>
<td>122</td>
</tr>
<tr>
<td>Desulfotomaculum nigrificans</td>
<td>16</td>
<td>81</td>
</tr>
<tr>
<td>Desulfovibrio vulgaris</td>
<td>140</td>
<td>5</td>
</tr>
<tr>
<td>Desulfovibrio gigas</td>
<td>159</td>
<td>10</td>
</tr>
<tr>
<td>Desulfovibrio desulfuricans</td>
<td>275</td>
<td>5</td>
</tr>
</tbody>
</table>

* Micromoles of Pi per minute per milligram. Values are divided by 2 (see the text).
† Micromoles of acetyl phosphate per minute per milligram.
**Fig. 1.** DEAE-cellulose chromatography of the 50% ammonium sulfate supernatant of Desulfovibrio vulgaris. Activities are indicated as units per milliliter, and dithionite was added to 25 mM.

**Fig. 2.** DEAE-cellulose chromatography of the 50% ammonium sulfate supernatant of Desulfotomaculum ruminis. Activities are indicated as units per milliliter, and dithionite was added to 25 mM.
coupled phosphorylation for growth on these substrates. In contrast, Desulfovibrio has an obligatory requirement for electron-transfer-coupled phosphorylation for growth on lactate plus sulfate (27). The bioenergetics and growth yields for Desulfitomaculum orientis and Desulfovibrio vulgaris growing on lactate plus sulfate are shown in Table 4. Both organisms generate two ATPs by substrate phosphorylation coupled to the oxidation of pyruvate. Desulfovibrio vulgaris utilizes both high-energy phosphates for the formation of APS, whereas Desulfitomaculum orientis utilizes only one high-energy phosphate by conserving the energy of the pyrophosphate bond. It is assumed that Desulfitomaculum orientis does not carry out electron transfer-coupled phosphorylation but that Desulfovibrio vulgaris gains three ATPs per sulfate reduced, as proposed by Badziong and Thauer (5). These considerations suggest that Desulfovibrio vulgaris has a net formation of three ATPs per sulfate reduced, whereas Desulfitomaculum orientis has a net ATP yield of one (Table 4). These differences should be reflected in the relative growth yields of the two types of organisms on lactate plus sulfate; as shown in Table 4, Desulfovibrio vulgaris produced three times as much cell material as Desulfitomaculum orientis when grown on lactate plus sulfate. The growth yield of Desulfovibrio vulgaris for these substrates was comparable to that previously reported (23). The data strongly indicate that under these growth conditions Desulfitomaculum orientis does not carry out electron-transfer-coupled phosphorylation.

**DISCUSSION**

Our results demonstrate that a basic difference exists between the bioenergetics of sulfate reduction in Desulfitomaculum and Desulfovibrio which involves the enzymes involved in the removal of pyrophosphate "armed during sulfate activation by ATP sulfurylase. Extracts of Desulfitomaculum orientis, Desulfitomaculum ruminis, and Desulfitomaculum nigrificans exhibit only low levels of PPase but do contain PP:acetate kinase. The level of this enzymatic activity is comparable to the level of PPase activity in extracts of Desulfovibrio. PP:acetate kinase was not detected in extracts of Desulfovibrio vulgaris, Desulfovibrio desulfuricans Norway 4, or Desulfovibrio gigas. The levels of activity found in extracts and the observation that both activities are reductant activated suggest that the enzymes serve analogous functions in the two groups of sulfate-reducing bacteria.

PP:acetate kinase has only been reported in extracts of E. histolytica (34), but its absence has been noted in a number of anaerobic microorganisms (44). The reaction has been studied largely in the direction of PP, formation rather than utilization, but the reaction was shown to be reversible. As the rate in the direction of acetyl phosphate formation was only 2% of the rate in the direction of PP, formation the enzyme does not appear to be particularly well suited for pulling the formation of APS by means of the utilization of PP,. The kinetics of acetyl phosphate formation need to be further investigated in this regard, particularly with respect to the effects of Mg (15, 33). Alternatively, the affinity of APS reductase for APS may be increased in Desulfitomaculum, and the ATP sulfurylase may be essentially pulled in the direction of PP, formation. In spite of these reservations, the level of activity of PP:acetate kinase observed in crude extracts indicates that this enzyme can function effectively in the utilization of PP.

By conserving the energy of the pyrophosphate bond, the three species of Desulfitomaculum are able to generate one ATP at the substrate level during growth on lactate plus sulfate as shown in the following reactions:

\[
2 \text{lactate} \rightarrow 2 \text{pyruvate} + 4e + 4H^+ \quad (6)
\]

\[
2 \text{pyruvate} + 2P_i \rightarrow 2 \text{acetyl phosphate} + 2\text{CO}_2 + 4e + 4H^+ \quad (7)
\]

\[
2 \text{acetyl phosphate} + \text{AMP} + 2\text{H}^+ \rightarrow \text{ATP} + 2 \text{acetate} \quad (8)
\]

\[
\text{ATP} + \text{SO}_4^{2-} + 8e + 8\text{H}^+ \rightarrow \text{S}^{2-} + \text{PP}_{i} + \text{AMP} + 3\text{H}_2\text{O} \quad (9)
\]

\[
\text{PP}_{i} + \text{acetate} \rightarrow \text{acetyl phosphate} + \text{P}_{i} \quad (4)
\]

**Table 4. ATP balances and growth yields for test strains grown on lactate plus sulfate**

<table>
<thead>
<tr>
<th>Strain</th>
<th>ATP formation and utilization*</th>
<th>Growth yield Y_{SO_4}^- b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Substrate coupled</td>
<td>Electron transfer</td>
</tr>
<tr>
<td>Desulfovibrio vulgaris</td>
<td>+2</td>
<td>+3</td>
</tr>
<tr>
<td>Desulfitomaculum orientis</td>
<td>+2</td>
<td>0</td>
</tr>
</tbody>
</table>

* Moles of ATP produced and utilized during the reduction of 1 mol of SO_4^{2-} to S^{2-} with lactate.

b Grams (dry weight) per mole of SO_4^{2-} reduced.
Acetyl phosphate + ADP → ATP + acetate

SUM: 2 lactate + ADP + P_i + SO_4^{2-} → 2 acetate + 2CO_2 + S^{2-} + ATP + 3H_2O

It is thus not necessary that these sulfate-reducing bacteria carry out electron-transfer-coupled phosphorylation for growth, and our data on relative growth yields indicate that Desulfotomaculum orientis does not generate ATP by electron-transfer-coupled phosphorylation during growth on lactate plus sulfate. This conclusion explains our failure to obtain sulfate reduction with H_2 as an electron donor for growth with sulfate. Growth on this substrate would require the presence of electron-transfer-coupled phosphorylation, and growth with formate may induce such a system. On the other hand, Desulfotomaculum ruminis does not grow well on formate and, on defined media, requires the presence of either lactate or alanine for growth (13). Clearly, the biochemistry and physiology of growth with formate and sulfate require further investigation.

Of the three types of c-type cytochrome found in Desulfovibrio, only cytochrome c_3 (M_r = 13,000) has been reported to exist in all species (19). Elegant information is available concerning the structure and oxidation-reduction properties of this unique multiheme cytochrome, but little information is available on its function. Evidence has been presented that it functions as a cofactor for hydrogenase in the reduction of ferredoxin, flavodoxin, and rubredoxin (6) and that it can function as a colodial sulfur reductase in Desulfovibrio desulfuricans Norway 4 but not in Desulfovibrio vulgaris (14). The fact that cytochrome c_3 is found only in Desulfovibrio suggests that this cytochrome is unique to respiratory sulfate reduction and that its major function will be found in the electron transfer pathway involved in the reduction of sulfite to sulfide.

Our observations also have some broader implications for the physiology and ecology of the sulfate-reducing bacteria. From the bioenergetic point of view, Desulfovibrio appears to be better adapted for growth on sulfate than Desulfitomaculum in that Desulfovibrio generates three times as much ATP during growth on lactate or ethanol plus sulfate. Thus, Desulfovibrio has a bioenergetic advantage in sulfate-rich environments, and this may account for the observation that marine species of Desulfotomaculum are rare (31). Bioenergetic considerations may also have relevance for the widespread distribution of Desulfovibrio and Desulfotomaculum in freshwater environments. Several species of Desulfovibrio have been shown to grow on lactate or ethanol in the absence of sulfate with a H_2-oxidizing methanogenic bacterium by the process of interspecies hydrogen transfer (10). Desulfotomaculum nigrificans is commonly found in thermophilic cellulose-methane enrichments and has been shown to grow in coculture with Methanobacterium thermoautotrophicum in the absence of sulfate (42). Both genera of sulfate-reducing bacteria are capable of growth by interspecies H_2 transfer and, in the absence of sulfate, would only obtain energy from substrate phosphorylation, two ATPs per methane produced. In this mode of growth, the two types of sulfate-reducing bacteria would be bioenergetically equivalent and presumably more competitive than in marine environments.

Two new genera of sulfate-reducing bacteria have been recently isolated (P. Widdel and N. Pfennig, personal communication) which utilize fatty acids as electron donors for sulfate reduction: Desulfofacter postgatei, which oxidizes even-numbered fatty acids, such as butyrate, to acetate; and Desulfofubus propionicus, which oxidizes odd-numbered fatty acids to acetate. Little is known concerning the pathways of oxidation, but assuming established pathways for the oxidation of butyrate and propionate to acetate, the presence of PPI:acetate kinase would allow these bacteria to generate net ATP from substrate phosphorylation. A new species of Desulfotomaculum has been reported (41) which is capable of oxidizing acetate to CO_2 with the concomitant reduction of sulfate to sulfide. The pathway of acetate oxidation is unknown, but conservation of the energy of the pyrophosphate bond may play an important role in the bioenergetics of this mode of growth.

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LITERATURE CITED


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