Discrimination between $^{34}$S and $^{32}$S during Bacterial Metabolism of Inorganic Sulfur Compounds

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Sulfur isotope effects during the oxidation of thiosulfate by Thiobacillus versutus were found to be negligible. This result is considered in relation to other oxidative and reductive processes to assess which reactions are most likely to control the isotopic compositions of sulfur compounds in microbial sulfureta.

Many processes in the Earth’s sulfur cycle are catalyzed by microorganisms. One useful approach to the study of these processes is based on determination of $^{34}$S/$^{32}$S ratios, which are significantly affected by certain microbial processes. Here we report studies of isotopic fractionations during the aerobic oxidation of thiosulfate by Thiobacillus versutus and summarize the isotope effects reported to occur during microbial oxidation of reduced inorganic sulfur compounds.

$T. \text{versutus}$ (formerly Thiobacillus A2) ATCC 25364 was grown at Martin Marietta Laboratories, Baltimore, Md., at 28°C aerobically on a rotary shaker operating at 180 rpm. The culture medium (20) contained Na$_2$S$_2$O$_3$ and NaHCO$_3$ serving as the energy and carbon sources, respectively; MgCl$_2$ replaced MgSO$_4$ as the magnesium source. Small-scale cultures were grown in 50 ml of the above medium in a 250-ml Erlenmeyer flask. To prepare an inoculum for the isotope fractionation experiments, cells from late-logarithmic phase, small-scale cultures were harvested by centrifugation, washed twice in distilled water, resuspended in 10 ml of growth medium, and used to inoculate 1 liter of growth medium in a 3-liter Erlenmeyer flask. The progress of growth was followed by monitoring the optical density at 600 nm.

Duplicate 15-ml samples were taken at frequent intervals. The cells were sedimented by centrifugation, and the supernatant fluid was filtered (0.2-µm Acrodisc filters; Gelman Sciences, Inc., Ann Arbor, Mich.) and stored frozen before analysis. Samples were shipped frozen on dry ice to Indiana University, thawed, and analyzed for sulfate and thiosulfate concentrations (18, 19). For isotopic analyses, sulfate was precipitated with BaCl$_2$ and then pelleted by centrifugation; the addition of hydrochloric acid to the pellet dissolved BaCO$_3$, leaving BaSO$_4$. Thiosulfate was oxidized with Na$_2$O$_2$ or, for intramolecular analyses, heated with silver nitrate to produce Ag$_2$S and SO$_3^{2-}$ from the outer sulfane and inner sulfonate positions, respectively (1). Isotopic analyses were performed as previously described (4), and $\delta^{34}$S values are reported relative to the isotopic composition of total thiosulfate, where $\delta^{34}$S = [(Rsample/Rthiosulfate) - 1] × 1,000 and $R = ^{34}$S/$^{32}$S. The total uncertainty (95% confidence limits) in the $\delta^{34}$S measurements was about ±0.4‰.

During autotrophic growth of $T. \text{versutus}$, thiosulfate was oxidized to sulfate (Fig. 1a), and cessation of growth was

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**TABLE 1. Isotope effects determined for oxidation of reduced sulfur compounds**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Oxidizing organisms or material</th>
<th>$\epsilon$ (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{H}_2\text{S} \rightarrow \text{S}^{2-}$</td>
<td>Photosynthetic bacteria</td>
<td>+2</td>
<td>3, 5, 8-10, 12, 13, 16</td>
</tr>
<tr>
<td>$\text{S}^{2-} \rightarrow \text{SO}_4^{2-}$</td>
<td>Photosynthetic bacteria</td>
<td>0</td>
<td>3, 5, 9</td>
</tr>
<tr>
<td>$\text{SO}_4^{2-} \rightarrow \text{S}^{2-} + \text{SO}_3^{2-}$</td>
<td>Chromatium vinosum</td>
<td>0</td>
<td>4, 8</td>
</tr>
<tr>
<td>$\text{SO}_3^{2-} \rightarrow \text{SO}_4^{2-}$</td>
<td>Chromatium vinosum</td>
<td>+5 (initial), -5 (final)</td>
<td>4</td>
</tr>
<tr>
<td>Aerobic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{H}_2\text{S} \rightarrow \text{S}^{2-}, \text{SO}_2^{2-}, \text{SO}_3^{2-}$</td>
<td>O$_2$, nonbiological</td>
<td>$-5^{a}$</td>
<td>7, 12, 13</td>
</tr>
<tr>
<td>$\text{H}_2\text{S} \rightarrow \text{S}^{2-}, \text{SO}_2^{2-}$</td>
<td>Thiothrix concretivorus</td>
<td>0 to -18</td>
<td>7, 8, 10</td>
</tr>
<tr>
<td>$\text{FeSO}_4 \rightarrow \text{SO}_2^{2-}$</td>
<td>Natural muds, thiothrix</td>
<td>0</td>
<td>17, 21</td>
</tr>
<tr>
<td>$\text{S}^{2-} \rightarrow \text{SO}_2^{2-}$</td>
<td>Various thiobacilli</td>
<td>0</td>
<td>6-10, 15, 17</td>
</tr>
<tr>
<td>$\text{SO}_3^{2-} \rightarrow \text{SO}_2^{2-}$</td>
<td>Various thiobacilli</td>
<td>0</td>
<td>2, 8, this study</td>
</tr>
<tr>
<td>$\text{SO}_3^{2-} \rightarrow \text{SO}_4^{2-}$</td>
<td>O$_2$, nonbiological</td>
<td>$-0.4$</td>
<td>4</td>
</tr>
</tbody>
</table>

* $\epsilon = 1,000 \ln \alpha$. Expressed in per mille, it is equal to the isotopic fractionation observed in initially formed products in simple unidirectional reactions. The best current estimate of $\epsilon$ from the listed references is given.

* A. Fry and W. Ruf, unpublished experimental work. The tabulated value is the isotope effect determined by measurement of the isotopic composition of unreacted sulfide.
approximately coincident with exhaustion of thiosulfate (cf. Fig. 1a and b). Little or no difference was observed between the isotopic compositions of sulfate and average thiosulfate (δsulfate = 0‰, Fig. 1c). Calculation of the isotope effect involved in the production of sulfate from thiosulfate was by previously described methods (14) and yielded $\epsilon = 0.4 \pm 0.4%$ (95% confidence limits) for combined data from three experiments (Fig. 1d) [note that the results summarized in this graph, although apparently widely scattered, range over less than two parts per thousand in $^{34}$S/$^{32}$S]. Analyses of the inner sulfonate and outer sulfane positions of thiosulfate showed significant isotopic differences between the two sulfur atoms: sulfonate $\delta^{34}$S = 4.4 ± 0.1‰ ($n = 3$), and sulfane $\delta^{34}$S = -4.2 ± 0.1‰ ($n = 6$). Slightly more rapid oxidation at the sulfonate position may be indicated since the sulfate formed initially tended to be slightly enriched in $^{34}$S (Fig. 1d, right side). Overall, however, the near-zero values observed throughout the experiments indicate that the sulfonate and sulfane positions were oxidized at very nearly equal rates and that there was essentially no isotope effect.

Summarized in Table 1 are results of prior measurements of sulfur isotope effects associated with bacterial oxidation of inorganic sulfur compounds. Previous studies of the oxidation of thiosulfate have involved anaerobic oxidation by purple photosynthetic bacteria of the genus *Chromatium* (4, 8) and aerobic oxidation by *Thiobacillus neapolitanus* C (results of Kelly et al., reported in reference 2). As in the present work, small or no isotope effects were found in the anaerobic studies, but larger fractionations were reported in the aerobic study. In that case, polythionates accumulated as intermediates, a phenomenon not observed in the present work. Consideration of relevant reports (2, 7, 10) suggests that larger fractionations are often associated with the formation of polythionates and that the fractionation observed by Kelly et al. was related to that process, not the oxidation of thiosulfate to sulfate. Accordingly, the "best" value reported in Table 1 for aerobic oxidation of thiosulfate is that observed in the present work.

Most of the isotope effects reported in Table 1 are small, ranging from about -5 to +2‰. The larger effect of -18‰ reported for the oxidation of $\text{H}_2\text{S}$ by *Thiobacillus concretivorus* has not been independently confirmed and was observed in experiments in which polythionates accumulated. Nonbiological oxidations of $\text{H}_2\text{S}$ and $\text{SO}_4^{2-}$ are also accompanied by small isotope effects of -5 to 0‰ (Table 1).

In contrast to the small isotope effects summarized in Table 1, fractionations associated with bacterial reduction of sulfate are typically larger, ranging between -15 and -40‰, although the complete range of values extends from near zero to <60‰ (2). We conclude that isotope effects associated with the reduction of sulfate will usually determine the isotopic contrasts between reduced and oxidized sulfur compounds in microbial systems. Exceptions to this generalization may occur when the rate of sulfide oxidation significantly exceeds that of sulfate reduction. Under such conditions, for example, during some intervals in natural diurnal cycles, the smaller but significant isotope effects occurring during anaerobic or aerobic oxidations of sulfide

*versus*.

(c) Isotopic composition of sulfate (see text). (d) Linearized plot ($f = \text{unconsumed fraction of reactant}$ (14)) of isotopic composition of product; the isotope effect is given by the slope, $\epsilon = 0.4 \pm 0.4%$. Data are pooled from three experiments.
(Table 1) could lead to a large change in the isotopic values of a small residual pool of reduced sulfur. Fractionation of sulfur isotopes in mixed cultures of anoxygenic photosynthetic and sulfate-reducing bacteria during light-dark cycles is currently under study in our laboratories.

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LITERATURE CITED


