Assimilatory Sulfur Metabolism in Marine Microorganisms: a Novel Sulfate Transport System in Alteromonas luteoviolaceus†

RUSSELL L. CUHEL,†† CRAIG D. TAYLOR, AND HOLGER W. JANNASCH
Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

Received 5 January 1981/Accepted 7 May 1981

The sulfate transport mechanism of a marine bacterium, Alteromonas luteoviolaceus, was unique among microorganisms in its extremely low affinity for the sulfate analog thiosulfate. Distinguishing characteristics included weak inhibition of sulfate transport by thiosulfate, inability to transport thiosulfate effectively, poor growth using thiosulfate as the sole source of sulfur, and a mild effect of the sulfhydryl reagent para-hydroxymercurobenzoate. In contrast, sulfate transport by a marine pseudomonad, Pseudomonas halodurans, was strongly inhibited by thiosulfate, and para-hydroxymercurobenzoate reversibly but completely blocked sulfate transport.

The close relationship between sulfur metabolism and protein synthesis in microorganisms (4, 17) has stimulated us to pursue the study of sulfate incorporation into protein as a measure of marine bacterial growth. However, studies of sulfur metabolism by marine bacteria are hampered by a large isotope dilution barrier imposed by the 25 mM sulfate concentration of seawater.

Sulfate transport systems in microorganisms exhibit a great degree of uniformity across phylogenetic boundaries, and a universal characteristic of the transport mechanism is the effective inhibition of sulfate uptake by a structurally related, more reduced sulfur compound, thiosulfate (1, 3, 5, 12, 14, 19, 20, 22, 23). Furthermore, thiosulfate is an excellent source of sulfur for the growth of microorganisms (3, 9, 11, 16, 17), and the reduced (sulfane) moiety is preferentially incorporated into the sulfur-containing amino acids relative to the sulfite moiety, or sulfate (6, 10). The highly predictable nature of the sulfate transport system led us to consider using [35S]-thiosulfate to competitively replace sulfate in marine bacterial uptake and metabolism. This concept was supported by kinetic studies of sulfate and thiosulfate uptake in a marine pseudomonad, Pseudomonas halodurans (3). The data demonstrated that both compounds were transported by the same system, but with a 10-fold higher affinity for thiosulfate.

During a survey of thiosulfate utilization by marine bacteria, we isolated Alteromonas luteoviolaceus, a distinctively pigmented bacterium which did not utilize thiosulfate as the sole source of sulfur for growth. Analysis of its sulfate transport system has revealed several unique features, including the absence of inhibition by the sulfhydryl reagent para-hydroxymercurobenzoate (pHMB), lack of thiosulfate transport, and extremely weak inhibition of sulfate uptake by thiosulfate. Comparative experiments with P. halodurans emphasize the peculiar nature of the A. luteoviolaceus transport system; other aspects of sulfate uptake and its regulation in these bacteria have been previously reported (3).

MATERIALS AND METHODS

Organisms and culture conditions. P. halodurans, A. luteoviolaceus, and conditions of growth and harvesting have been previously described (3).

Sulfate transport assay. The methods described by Cuvel et al. (3) were used, except that the assay mixture was increased to 25 ml for experiments involving pHMB. Anaerobiosis was prevented in these extended assays by use of small Erlemeyer flasks with stirring bars.

Other methods. The methods, chemicals, and sources of radioisotopes described in reference 3 were used throughout. The source of pHMB was Sigma Chemical Co. (St. Louis, Mo).

RESULTS

Thiosulfate produced no significant concentration-dependent inhibition of sulfate transport in A. luteoviolaceus until present at 50 times the sulfate concentration in the assay mixture (Table 1). For comparison, thiosulfate competitively inhibited sulfate uptake in P. halodurans (Fig. 1), with the inhibition constant, K', less than one-tenth the Ks for sulfate uptake (3).
Table 1. Thiosulfate inhibition of sulfate transport by A. luteo-violaceus

<table>
<thead>
<tr>
<th>Thiosulfate concn (μM)</th>
<th>Sulfate uptake rate (pmol of SO₄ per 10⁸ cells per min)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>57.6</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>54.9</td>
<td>5</td>
</tr>
<tr>
<td>100</td>
<td>57.6</td>
<td>0</td>
</tr>
<tr>
<td>500</td>
<td>49.8</td>
<td>14</td>
</tr>
<tr>
<td>1,000</td>
<td>50.8</td>
<td>12</td>
</tr>
<tr>
<td>5,000</td>
<td>23.7</td>
<td>59</td>
</tr>
</tbody>
</table>

*Sulfate transport at a final concentration of 100 μM (19 dpm/pmol) was assayed as previously described (3). The cell density was 2.4 × 10⁸/ml.

Fig. 1. Lineweaver-Burk plot of competitive inhibition of sulfate uptake by thiosulfate in P. halodurans. A cell suspension of 3.0 × 10⁸/ml was used for assay (3); the Kᵢ was 16.6 μM.

Cell suspensions of A. luteo-violaceus which had been starved for sulfur for 2 h to enhance transport activity did not take up detectable amounts of [³⁵S]thiosulfate until a concentration of 500 μM, the highest test, was reached. At this concentration, the observed uptake rate of 4 pmol of S₂O₃²⁻ per 10⁸ cells per min was much less than the 77 pmol per 10⁸ cells per min found for P. halodurans under the same conditions. Finally, when inoculated into complete medium with 1 mM thiosulfate as the sole source of sulfur, A. luteo-violaceus grew with a lag time of 6 to 7 days relative to the 1 mM sulfate control, but then grew at a similar rate. When thiosulfate-grown cells were reincubated into fresh medium, the lag was again observed in thiosulfate-containing medium, although the sulfate-supplemented culture grew normally, indicating that induction of enzymes specific for thiosulfate metabolism was not involved in promoting growth. A. luteo-violaceus grows at sulfate concentrations less than 10 μM, so a slow rate of thiosulfate oxidation in the medium or sulfate contamination of thiosulfate could have been responsible.

The major difference between thiosulfate and sulfate is the substitution of a reduced sulfur atom for one of the four equivalent oxygen atoms of sulfate. It is therefore probable that the higher affinity of P. halodurans for thiosulfate is due to the chemical reactivity of the sulfane moiety of thiosulfate with a sulfhydryl group in the transport enzyme. Sulfate transport systems are known for their reversible sensitivity to the sulfhydryl reagent pHMB (13, 21), and Fig. 2 shows that pHMB exerted a rapid and complete inhibition of sulfate uptake in P. halodurans which was partially reversed by the addition of the reducing agent dithiothreitol. In contrast, pHMB had very little effect on sulfate uptake by A. luteo-violaceus (Fig. 3). The extent of inhibition was not increased with exposure time or preincubation, but was relieved by dithiothreitol. These data suggest that the mildly reduced sulfate uptake was not directly due to the action of pHMB, but rather to a secondary effect such as transport of glutamate, the energy source in these experiments.

Fig. 2. Effects of 10 μM pHMB on sulfate uptake by P. halodurans. Sulfate (100 μM; 40 dpm/pmol) was added either simultaneously (t₀) or after a 2-min preincubation with pHMB (t₂). Dithiothreitol (DTT; 100 μM) was added at the arrow to one control and to one sample preincubated with pHMB. The cell density was 3.2 × 10⁸/ml.
DISCUSSION

The A. luteo-violaceus sulfate transport system is most unusual in the limited effect of thiosulfate on sulfate transport and lack of growth on this compound. When sulfate and thiosulfate are present in equimolar amounts, sulfate transport is inhibited at least 50% in most organisms (1, 12, 14, 20, 22), and frequently much more (5, 17). When tested, these sulfate transport systems are also sensitive to pHMB, with rapid and nearly complete blockage of uptake (13, 21). The action of the sulphydryl reagent and the preference for thiosulfate in transport suggests that the reduced sulfur moiety of thiosulfate is an important component in the mechanism of its transport. The kinetics of sulfate and thiosulfate uptake in P. halodurans (3) are consistent with other reports in these respects. Sulfate transport in Salmonella typhimurium is sensitive to sulphydryl reagents and thiosulfate, but the binding protein lacks these characteristics and contains no cysteine residues (14). Thus it is likely that the unusual feature of the A. luteo-violaceus sulfate transport system is localized in the carrier protein rather than in a sulfafacialized binding site.

The singular nature of the Alteromonas sulfate transport system presents an interesting possibility for marine microbial ecologists. Due to the very limited work on taxonomy of marine bacteria, most studies of bacterial distribution in the ocean are confirmed to organisms possessing distinctive traits such as bioluminescence (18), but few species have been found which are so readily identified. The characteristic pigment produced by A. luteo-violaceus, violacean, is identical to the principal pigment of Chromobacterium sp. (2), for which a marine species has been reported (8). The similarity in pigmentation, narrow nutritional capability, proliferation of extracellular hydrodases, and morphology has led Gauthier (7) to suggest that Chromobacterium marinus (8) and A. luteo-violaceus are indeed the same organism. The original strain of C. marinus was lost, however, so the issue has not been resolved. The unusual response of A. luteo-violaceus to thiosulfate is simple to test and may provide an additional taxonomic tool for identifying the organism in natural population enrichments. We have isolated A. luteo-violaceus from diverse habitats in the northwestern Atlantic Ocean, often from surfaces such as Sargassum weed and fish. Gauthier's organisms were isolated from the Mediterranean Sea. Its peculiar sulfate transport system may thus aid in a comprehensive investigation of the distribution of this widespread marine bacterium, which would be of interest to marine microbial ecologists.

ACKNOWLEDGMENTS

This work was supported by National Science Foundation grants OCE77-12172, OCE79-19178, and OCE79-19264. Further support for R.L.C. was provided by the Education Department of the Woods Hole Oceanographic Institution.

LITERATURE CITED


