Uptake Rates of Oxygen and Sulfide Measured with Individual Thiomargarita namibiensis Cells by Using Microelectrodes

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The sulfur bacterium Thiomargarita namibiensis is a close relative of the filamentous sulfur bacteria of the genera Beggiatoa and Thioploca. It was only recently discovered off the Namibian coast in fluid sediments rich in organic matter and sulfide (15). The large, spherical cells of Thiomargarita (diameter, 100 to 300 μm) are held together in a chain by mucus that surrounds each cell (Fig. 1). Most of the cell volume is taken up by a central vacuole in which nitrate is stored at concentrations of up to 800 mM. The ability to accumulate nitrate is also found in larger, marine species of Beggiatoa (8) and Thioploca (3). The latter have been shown to use nitrate as an electron acceptor for the oxidation of sulfide to elemental sulfur and then to sulfate while they reduce nitrate to ammonia (13). Both Beggiatoa and Thioploca spp. show a phobic reaction towards oxygen even at low concentrations (5, 10). Higher oxygen concentrations in the bottom water (5 to 35 μM), when they occur off the Chilean coast during winter or El Niño events, dramatically reduce the Thioploca population (16). In contrast to this, Thiomargarita cells survive exposure to oxygen even at the concentrations of air saturation (15), although the bottom water overlying the sediments off Walvis Bay is usually anoxic.

Because Thiomargarita cells are not motile, the only way that the cells can come into contact with water containing nitrate is during intervals when the sediment gets resuspended in the water column. This can happen, for example, due to large sediment outgassings of methane, which occur regularly in the area inhabited by Thiomargarita (2, 17). During these events the highly fluidized sulfidic mud containing Thiomargarita cells may get mixed with oxygenated water containing nitrate. The purpose of this study was to investigate whether the cells merely survive such exposure to oxygen or whether Thiomargarita cells can use oxygen as an electron acceptor in addition to nitrate for oxidation of sulfide. Like the nitrate-storing species of Beggiatoa and Thioploca, Thiomargarita spp. have not been isolated yet in pure culture. Nevertheless, cells may be kept alive and growing in their native sediment for years. As Thiomargarita cells are not motile, it is not possible to draw conclusions about their physiology by observing chemotactic behavior, as has been done successfully with Beggiatoa and Thioploca filaments (5, 10). However, because of the large size of Thiomargarita cells, they develop, around individual cells, measurable gradients of oxygen and sulfide that can be used for calculating uptake rates of oxygen and sulfide. Thus, the physiological reactions of individual cells to changes in oxygen and sulfide concentrations can be directly observed by observing changes in the uptake rates.

Experiments. The cells used for the experiments in this study were collected off Namibia during a cruise of the German RV Poseidon in May 1999 and were kept in their natural sediment at 5°C for more than 1 year. At approximately 3-month intervals the overlying water was removed and the sediment was resuspended several times in seawater enriched with nitrate (1 mM), which induced growth of Thiomargarita cells. During this treatment cells were exposed to oxygen. The experiments were conducted in a square polycarbonate chamber (7 by 7 by 7 cm) containing 250 ml of artificial seawater (36.4 g of NaCl per liter, 1 g of CaCl2 per liter, 0.5 g of K2HPO4 per liter, 0.1 g of NaH2PO4 per liter; pH 7.3). The chamber was capped with a movable plate containing two holes for microelectrode access (Fig. 1). Thiomargarita cells were washed in medium and transferred to the chamber by sucking them up in the tip of a Pasteur pipette. After the cells were placed in the experimental chamber, acetate was added to a final concentration of 10 μM. The chamber was continuously flushed with either air or argon to control the oxygen concentration. Sulfide was added to the medium by passing the gas through a bottle containing 100 ml of carbonate buffer (pH 9.3) and sulfide at a high concentration (100-fold higher than the desired final concentration in the experimental chamber). Thus, the gas was supplemented with H2S at the desired concentration before it was bubbled through the experimental chamber. After a stable concentration of sulfide was established, as measured with an H2S microsensor, samples (1 ml) were taken, and the exact sulfide concentration was determined spectrophotometrically (1). The bubbling was adjusted so that the liquid in the chamber was well mixed and only a thin diffusive boundary layer (DBL) (approximately 140 μm) developed around the cells. The DBL around the cells (diameter, 220 μm) was found by moving the microsensor in 10-μm intervals away from the cell until no change in concentration was found. To measure fluxes of ox-
During the experiment, the electrode was moved 500 μm to place it directly at the cell surface. Several times, the electrode was lowered to a distance of ca. 1 cm from the bottom of the chamber (Fig. 1). By using a preparation needle, the two ends of the chain were placed into the V-shaped ends of the platinum wires. This design allowed three-dimensional diffusion toward the cell investigated. Vertical profiles of oxygen or sulfide were measured through the DBL of the cells at 10-μm intervals by using a Clark-type oxygen microelectrode with a guard cathode or a sulfide microelectrode. The gradients through the DBL of the cells were found to be almost linear; therefore, the total flux towards the cell could be calculated by multiplying the linear flux \( J = D \times dC/dr \), where \( J \) is the flux, \( D \) is the diffusion coefficient, \( dC \) is the change in concentration between two points, and \( dr \) is the distance between these two points) by the surface area of the cell (diameter, 220 μm). To calculate the surface area involved in uptake, either the surface of a sphere \( (4\pi r^2) \) or the surface of the side of a cylinder \( (2\pi rh) \), where \( r \) is the radius and \( h \) is the height) could be assumed. In our case, the height of the cylinder was twice the radius \( (h = 2r) \), so the two assumptions gave the same value. During the experiment whose results are shown in Fig. 3, the cells were lying on the bottom of the experimental chamber. The oxygen electrode was placed directly at the cell surface. Several times during the experiment, the electrode was moved 500 μm up and down to ensure that the bulk concentration of oxygen remained unchanged. In order to prove that oxygen and sulfide gradients resulted from physiological activity of the cells, selected active cells were exposed for 1 min to pure ethanol and returned to the chamber. These cells showed no gradients of oxygen or sulfide towards the cell surface. During this short exposure, the cells were killed, but the internal sulfur inclusion did not dissolve. Also, addition of methanol to a final concentration of 1% in the medium led to immediate disappearance of sulfide and oxygen gradients around initially active cells. The abiotic oxidation of sulfide with oxygen is a slow process that occurs with half times between 0.4 and 65 h (9). As we flushed the medium continuously with both gases, this process should not have played a major role in these experiments.

**Addition of acetate.** In initial experiments, freshly added cells showed pronounced gradients of oxygen and sulfide that slowly disappeared within 1 or 2 h. Only after sodium acetate was added to the medium at a final concentration of 10 μM did the oxygen gradients remain stable for at least 2 days, even when no sulfide was present in the medium. Further addition of 10 μM acetate did not enhance the oxygen flux towards the cells (data not shown). If the 10 μM acetate were consumed by the cell and used as an electron donor, this result would have corresponded to a maximum acetate flux of 6.5 pmol cm\(^{-2}\) s\(^{-1}\) and would have increased the oxygen flux by 13 pmol cm\(^{-2}\) s\(^{-1}\) \((\text{CH}_3\text{COOH} + 2 \text{O}_2 \rightarrow 2 \text{CO}_2 + 2 \text{H}_2\text{O})\). As the oxygen flux was stabilized at only 1.1 pmol cm\(^{-2}\) s\(^{-1}\) and not increased even by the first addition of acetate, it is likely that the *Thiomargarita* cells in this experiment depended on acetate only as a carbon source and not as an electron donor. Acetate has also been shown to stimulate the sulfide uptake of *Thioploca* spp. (13) and the thiosulfate uptake of *Thiothrix* spp. (12) and can be used as a supplemental sole carbon source by lithotrophic marine *Beggioa* strains (4). Nevertheless, the possibility that *T. namibiensis* grows autotrophically cannot be ruled out by the results of these experiments, because the special setup, which was designed to measure sulfide uptake in the presence of minimum oxygen concentrations and with a stable pH, did not allow bicarbonate-carbonate to be present in the medium. To maintain very low oxygen concentrations, it was necessary to bubble the medium constantly with argon, which would have stripped CO\(_2\) from the medium.

**Use of oxygen.** The presence of sulfide in the medium clearly enhanced oxygen uptake (Fig. 2A), and likewise, sulfide uptake by the cells was enhanced by oxygen (Fig. 2B). These results suggest that *Thiomargarita* cells not only are able to survive exposure to oxygen but also may use oxygen as an electron acceptor. Addition of nitrate to the medium had no effect on the oxygen uptake. As judged by the sulfide uptake rates, the cells remained physiologically active even under oxygen concentrations close to saturation. This indicates that unlike *Beggiatoa* spp. (7, 10, 11), *T. namibiensis* is not obligately microaerophilic. The maximum sulfide flux was 7.5 pmol cm\(^{-2}\) to 15.5 pmol cm\(^{-2}\).
sulflde oxygen concentrations that are low (100 μM) with gas, the maximum uptake rates occurred at sulflde by internally stored nitrate. Addition of oxygen increased the cation of -obtained with cells lying on the bottom of the chamber, but quantification of fluxes from these profiles by using Fick’s law may have led to overestimates and were therefore not included in the data set.

The results of our experiments show that even though T. namibiensis cells store high concentrations of nitrate, they can take up oxygen in the presence or absence of nitrate. As the oxygen uptake is greatly stimulated by the presence of sulfide and vice versa, oxygen seems to be used as an electron acceptor for the oxidation of sulfide, even though the sediment in which these organisms are found is permanently anoxic. T. namibiensis is found are permanently anoxic.

Implications. The results of our experiments show that even though T. namibiensis cells store high concentrations of nitrate, they can take up oxygen in the presence or absence of nitrate. As the oxygen uptake is greatly stimulated by the presence of sulfide and vice versa, oxygen seems to be used as an electron acceptor for the oxidation of sulfide, even though the sediments in which T. namibiensis is found are permanently anoxic.
might be the major times of energy gain, whereas nitrate might be used primarily to survive the time between sediment suspension events.

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REFERENCES