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

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Received 25 March 2002/Accepted 31 July 2002

Gradients of oxygen and sulfide measured towards individual cells of the large nitrate-storing sulfur bacterium Thiomargarita namibiensis showed that in addition to nitrate oxygen is used for oxidation of sulfide. Stable gradients around the cells were found only if acetate was added to the medium at low concentrations.

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-
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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 \( \mu \text{M} \) did the oxygen gradients remain stable for at least 2 days, even when no sulfide was present in the medium. Further addition of 10 \( \mu \text{M} \) acetate did not enhance the oxygen flux towards the cells (data not shown). If the 10 \( \mu \text{M} \) 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 Thiotrix spp. (12) and can be used as a supplemental or sole carbon source by lithotrophic marine Beggiatoa 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 \( \text{pH} \), 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\(^{-2}\) s\(^{-1}\).

FIG. 1. Experimental setup. A chain of T. namibiensis cells was placed in a chamber with 250 ml of artificial seawater. The oxygen concentration in the medium was controlled by bubbling the medium with either air or argon. Sulfide was added by prior bubbling through a wash bottle containing a concentrated solution of sulfide. To ensure three-dimensional diffusion towards the cells, the chain of cells was elevated above the bottom of the chamber by clamping it between two platinum wires. One microelectrode (either oxygen or sulfide) was used to measure gradients towards the cells, while the other recorded the concentration of oxygen or sulfide in the bulk medium. The inset shows an example of oxygen flux without sulfide in the medium.
sulflde oxygen concentrations that are low (100 μM) with gas, the maximum uptake rates occurred at sulfur (H9262). It seems that with the rigorous stirring resulting from bubbling by internally stored nitrate. Addition of oxygen increased the concentration (Fig. 2B); likewise, the oxygen uptake (pmol cm−2 s−1) of Thiomargarita 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 observed sulfide flux under anoxic conditions (approximately 5 pmol cm−2 s−1) (Fig. 2B) must have been supported by internally stored nitrate. Addition of oxygen increased the sulfide flux by ca. 2.5 pmol cm−2 s−1. Thus, even though the sulfide flux could be significantly stimulated by oxygen, reduction of nitrate was still the more important process for sulfide uptake.

**Cell response to sulfide under aerobic conditions.** It was possible to monitor the reaction of an individual cell to pulses of sulfide in the medium by placing the oxygen electrode directly at the cell surface. A decline in the oxygen concentration represented higher uptake rates, and an increase indicated lower rates of oxygen uptake (Fig. 3). The response of the cell could be observed with very high temporal time resolution, but only relative changes in the uptake rate were visible. The fluxes could be obtained only from steady-state profiles. Two sequential pulses of sulfide (60 and 120 μM) both resulted in a sharp drop in the oxygen concentration, followed by an increase and then a decrease in the oxygen tension at the cell surface (Fig. 3). In both cases the minimum concentration at the cell surface was reached at sulfide concentrations of around 20 μM (24 and 18 μM, respectively). Together with the finding that oxygen uptake rates did not increase with sulfide concentrations above 37 μM, this suggests that under our experimental conditions Thiomargarita namibiensis reached maximum sulfide fluxes at a sulfide concentration of around 20 μM even though it tolerates much higher concentrations. After both sulfide pulses the oxygen concentration at the cell began to rise again after the sulfide concentration dropped below approximately 9 μM, indicating that this sulfide concentration was too low to support maximal oxygen uptake rates. The oxygen concentration returned to the former value only 9 h after sulfide was completely removed from the medium (Fig. 3). Possibly, the accumulation of elemental sulfur under aerobic conditions with sulfide present led to the long-term effect of the sulfide pulses on the uptake rate of oxygen. A similar experiment in which oxygen was added under anoxic, sulfide conditions resulted in a single drop and rise in the sulfide concentration at the cell surface (data not shown).

**Implications.** The results of our experiments show that even though Thiomargarita 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 Thiomargarita namibiensis is found are permanently anoxic. These observations suggest that even though most of the time Thiomargarita namibiensis cells survive in sediments containing high sulfide concentrations with internally stored nitrate as their sole electron acceptor, they may be physiologically most active during times when the sediment is suspended. Suspension of sulfidic mud in oxic seawater should provide the cells with the opportunity to oxidize sulfide with oxygen. Even though such events may be relatively infrequent, the rapid response to oxygen and the relatively high uptake rates suggest that these short periods
might be the major times of energy gain, whereas nitrate might be used primarily to survive the time between sediment suspension events.

This work was financed by the Max Planck Society.

We thank Anja Eggers, Gabi Eckert, and Ines Schröder for construction and help with the use of microelectrodes, Helle Ploug for help with calculations, and Doug Nelson for editing and for many helpful suggestions to improve the manuscript.

REFERENCES