Bacterial Sulfate Reduction Above 100°C in Deep-Sea Hydrothermal Vent Sediments

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The currently known upper temperature limit for growth of organisms, shared by a number of archaebacteria, is 110°C. However, among the sulfate-reducing bacteria, growth temperatures of greater than 100°C have not been found. A search for high-temperature activity of sulfate-reducing bacteria was done in hot deep-sea sediments at the hydrothermal vents of the Guaymas Basin tectonic spreading center in the Gulf of California. Radiotracer studies revealed that sulfate reduction can occur at temperatures up to 110°C, with an optimum rate at 103° to 106°C. This observation expands the upper temperature limit of this process in deep-ocean sediments by 20°C and indicates the existence of an unknown group of hyperthermophilic bacteria with a potential importance for the biogeochemistry of sulfur above 100°C.

Because of the abundance of sulfate in the ocean, its bacterial reduction to sulfide represents the main terminal pathway during the anaerobic mineralization of organic matter in the seabed (*1, 2*). Most reduction occurs at temperatures of 0° to 25°C. Extensive reduction of sulfate is also observed in marine hydrothermal systems associated with volcanism or with tectonic activity at ocean ridges (*3*). Because of favorable substrate competition and the unlimited availability of sulfate (*4–6*), as well as by inference from data obtained in colder sediments, bacterial sulfate reduction seems of greater quantitative importance than methanogenesis or sulfur (S°) reduction. It is difficult to distinguish between bacterial and thermolytic reduction of sulfate in geothermal systems, and only abiological reduction has generally been assumed to occur at temperatures above 100°C. Although organisms growing at temperatures above 100°C have been isolated from hot anoxic, marine environments, they have metabolisms that reduce CO_2 (methanogenic) or sulfur (S°) (*7–10*). Eubacteria that reduce sulfate do not grow at temperatures above 85°C (*11*), although some strains of sulfate-reducing archaebacteria of the genus *Archaeoglobus* grow at temperatures up to 90° to 95°C (*12, 13*). In this report, we present evidence that bacterial sulfate reduction may occur at temperatures up to 110°C in hot sediments around deep-sea hydrothermal vents.

Temperatures in the organic-rich sediments in the Guaymas Basin at a water depth of 2000 m increase from 3°C at the sediment surface to 120°C over a few tens of centimeters at local hot spots where hot pore fluids emanate (*14–16*). During dives with the research submersible *Alvin*, we collected sediment cores from several hot spots that could be visibly discerned from precipitates of black iron sulfide and the shimmering (temperature schlieren effect) of the overlying seawater. In the ship's laboratory, the cores were cut into 5-cm sections that were stored separately in gas-tight sterile plastic bags at 5°C. We measured the temperature dependence of sulfate reduction a few weeks later under controlled conditions in our institute's laboratory using ^{35}S-labeled sulfate (*17, 18*).

In samples obtained from increasing depths in the sediment, a gradual shift toward higher temperature optima of sulfate reduction was observed in accordance with the higher temperatures measured in situ. In near-surface sediment (0 to 5 cm, Fig. 1), only mesophilic and moderately thermophilic activity was found, with optima around 30° and 60°C, respectively. Beyond the main peak of extremely thermophilic sulfate reduction with optima between 80° and 90°C in the sections 10 to 20 cm deep, a distinct second peak was observed with an optimum at about 105°C (Fig. 1). The in situ temperatures measured at these sediment depths were in the range of 80° to 128°C. There was a distinct minimum of the sulfate reduction activity at 95° to 100°C between the two peaks. This minimum may indicate that there are two clearly different populations of bacteria in the sediment. The upper temperature limit of sulfate reduction in these samples must be slightly beyond 110°C. Because there was no activity at 118°C, we exclude the possibility that chemical sulfate reduction was occurring in our experiments. The maximal reduction rates measured in these samples, 19 to 61 μM SO_4^{2-} per day, are extremely high for deep-sea sediments and are comparable to those commonly found in coastal environments (*1*).

During the relatively long incubation times of 1 to 7 days, bacterial growth might have occurred and the proportions between populations of different temperature-adapted groups might have been affected. If such changes did occur, they clearly did not result in a continuum of temperature responses. This observation agrees with similar findings of two distinct temperature-adapted groups of sulfate-reducing bacteria in coastal marine sediments with activity optima around 30° and 60°C, respectively (*19*).

The sources of energy and carbon for these apparently new organisms are not known.

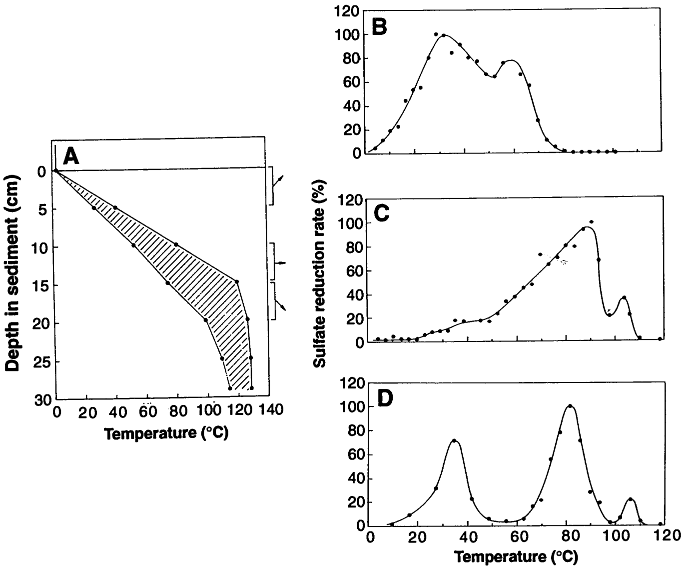

Fig. 1. Temperature gradient and temperature dependence of bacterial sulfate reduction in sediment from the hydrothermal vent field of Guaymas Basin. (**A**) Sediment temperature increased steeply from 3°C at the surface to >100°C at a depth of 15 to 20 cm. Different temperature groups of sulfate reducers are evident, including previously known hyperthermophiles with optima at 80° to 90°C and a novel group with an optimum >100°C. Sulfate reduction is expressed as percent of the maximum rate for each depth interval. The 100% activities for the three depth intervals [(**B**) 0 to 5 cm, (**C**) 10 to 15 cm, and (**D**) 15 to 20 cm] were 61, 24, and 19 μM SO_4^{2-} day^{-1}, respectively. Sediment temperature profiles at the sampling site were measured on several occasions by a thermistor probe operated by the mechanical arm of *Alvin*.

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The marine sediments of the Guaymas Basin are unusually rich in organic matter ($\sim$2% organic carbon), mostly of planktonic origin (*20*). Furthermore, the presence and vertical distribution of acetate and propionate in the Guaymas Basin hot sediments indicate that these organic acids are thermocatalytically produced from organic matter in deeper sediment layers at temperatures greater than 200° to 300°C. They appear to be consumed only in the top sediment layers where the temperatures fall below 110° to 120°C (*21*). Hydrothermally generated methane as well as aliphatic and aromatic hydrocarbons also rise from deeper layers by pore fluid transport and are abundant in the surface sediment (*22*). Although mesophilic use of C_{12} to C_{18} straight-chain aliphatics has been identified as a potential source of substrates for bacteria that reduce sulfate (*23*), the metabolic availability of hydrocarbons for anaerobic bacteria at higher temperatures has not yet been investigated.

It has not been uncommon to detect a microbial process before the isolation and description of the responsible organism. Yet, it was unusual that the first discovery of sulfate reduction at 90°C in the hot seabed of Guaymas Basin (*15*) was immediately followed by the isolation from the same sediment material of *Archaeoglobus profundus*, a sulfate-reducing bacterium growing in temperatures up to 90°C (*13*). Attempts to isolate the organisms growing at temperatures >100°C have so far been unsuccessful (*24*). The radiotracer technique we applied for measuring sulfate reduction at temperatures >100°C is sensitive and free from temperature-dependent artifacts, such as isotopic exchange or chemical reduction, within the temperature interval studied here. Enrichment culture and ultimate isolation of the presumed organisms are, in contrast, more uncertain and unpredictable without knowledge of substrate specificity and other growth requirements. As long as pure culture evidence for the existence of the bacteria is lacking, the radiotracer technique is thus an effective and reliable alternative to detect this microbial process.

The metal sulfides at the mid-oceanic ridges are by far the largest recent deposits on the global scale (*3*). Our results expand the temperature tolerance of bacterial sulfide production to a range where it approaches the lower temperature limit of thermolytic sulfate reduction (*25*). At such temperatures, this microbial process becomes important for an interpretation of the formation of sulfide deposits and their sulfur isotope distribution.

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17. Sediment sampled and collected at depth intervals of 5 cm from a core 15 cm wide by *Alvin* was used to measure the temperature dependence of sulfate reduction. Sediment was mixed in a 1:2 ratio with anoxic, artificial seawater, and 10-ml subsamples were dispensed under N_2 into test tubes and injected after temperature equilibration with 2 to 20 μCi of $^{35}SO_4^{2-}$. The tubes were incubated at more than 30 different temperatures in a stable temperature gradient of 3° to 120°C (±0.5°C) for 1 day (sample from 0 to 5 cm) or 7 days (sample from 10 to 15 and 15 to 20 cm). At temperatures >100°C, tubes were sealed by screw caps and thus attained equilibrium vapor pressure of >1 bar. Sulfate reduction rates were determined from the formation of ^{35}S-labeled, reduced sulfur as analyzed by the single-step chromium reduction technique (*18*). Although incubations were done at around 1 bar rather than at in situ pressure, a pressure release from 2000 m seems generally not to change metabolic activity of heterotrophic bacteria significantly (*26, 27*).
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Downstream Fining by Selective Deposition in a Laboratory Flume

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There has long been debate about the relative importance of abrasion versus selective deposition of the coarsest clasts in causing downstream fining of sediment in river systems. Although high fining rates observed in many natural rivers seem to require strong selective deposition, the ability of selective deposition to produce downstream size sorting has never been measured under controlled conditions. In an experiment using a long flume and a poorly sorted, bimodal gravel feed, downstream fining was produced by a factor of 1.3 in median size and 1.8 in 90th percentile size, over a distance of 21 meters. The experimental conditions rule out abrasion effects. Selective deposition appears to be a natural consequence of the transport and deposition of sufficiently poorly sorted or bimodal gravels and appears to be capable of accounting for fining rates observed in natural gravel rivers.

Going downstream most natural rivers become finer grained, higher in discharge, gentler in slope, and more sinuous. These changes have important effects on vegetation, flood characteristics, ecological habitats, and so forth. Although there has been some debate as to which of these effects are primary and which are secondary, it is clear that downstream changes in grain size and discharge must be among the basic driving factors. There are two broad explanations of the observed downstream decrease in grain size (downstream fining): abrasion, a nonconservative mechanism that converts large clasts into smaller ones; and selective deposition of the coarser clasts, in which the sizes of individual clasts are conserved and downstream fining results from a sedimentary fractionation process. Although it has long been recognized that selective

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