Large Sulfur Bacteria and the Formation of Phosphorite

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Supporting Online Material

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Large Sulfur Bacteria and the Formation of Phosphorite

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Phosphorite deposits in marine sediments are a long-term sink for an essential nutrient, phosphorus. Here we show that apatite abundance in sediments on the Namibian shelf correlates with the abundance and activity of the giant sulfur bacterium *Thiomargarita namibiensis*, which suggests that sulfur bacteria drive phosphogenesis. Sediments populated by *Thiomargarita* showed sharp peaks of pore water phosphate (≤300 micromolar) and massive phosphorite accumulations (≥50 grams of phosphorus per kilogram). Laboratory experiments revealed that under anoxic conditions, *Thiomargarita* released enough phosphate to account for the precipitation of hydroxyapatite observed in the environment.

The formation of phosphorites in marine sediments is a major long-term sink for phosphorus, removing it from the biosphere. The initial step in phosphorite formation is the precipitation of phosphate-containing minerals, for example, hydroxyapatite, followed by many other processes such as sediment transport, winnowing, and re-crystallization (*1*, *2*). A fundamental problem in explaining massive phosphorite deposits has been identifying mechanisms that can concentrate pore water phosphate enough to drive spontaneous precipitation of phosphorus minerals. Here we suggest a new mechanism, the episodic release of phosphate into the anoxic sediment by an abundant benthic bacterium that is specially adapted to survive under both oxic and anoxic conditions. *Thiomargarita* periodically contacts oxic bottom water in order to take up nitrate, and it survives long intervals of anoxia with nitrate stored internally (*3*). The phosphate uptake from different sources occurs when *Thiomargarita* forms thick mats at the sediment surface or is suspended in the oxic water column.

The giant sulfur bacterium *Thiomargarita namibiensis* occurs in high biomass in surface sediments off the coast of Namibia (*3*). Like its close relatives *Beggiatoa* spp. and *Thioploca* spp., this bacterium gains energy by oxidizing sulfide, which accumulates in anoxic marine sediments as a result of the degradation of organic matter by sulfate-reducing bacteria. The production of sulfide is directly proportional to the amount of organic carbon in the sediment, thus these large sulfide-oxidizing bacteria are abundant in highly productive upwelling areas, where the flux of organic material to the sea floor is high. *Thiomargarita* and *Beggiatoa* dominate sediments beneath the Benguela upwelling area off Namibia (*3*), whereas *Thioploca* dominates sediments off the South American west coast (*4*) and in the Arabian Sea (*5*). In all of these areas, modern phosphorite formation has been reported (*1*, *6*). All of these sulfur bacteria species contain large amounts of intracellular polyphosphates, which we found by staining cells specifically for polyphosphate with toluidine blue (*7*, *8*). Also, these bacteria show electron-dense inclusions (*3*, *9*, *10*), which is a typical appearance of polyphosphate.

During an expedition with the German research vessel *Meteor* off the coast of Namibia in March 2003, we found high pore water phosphate concentrations (*7*) of up to 300 μM in sediments that were densely populated by *T. namibiensis* (Fig. 1A). The sharp phosphate peaks that were observed in sediments were restricted to a narrow sediment horizon (about 3 cm thick), which corresponded to the depths where *T. namibiensis* was most abundant (Fig. 1C). Because of the high phosphate concentrations, active formation of phosphorite occurred in this thin zone as indicated by the large amounts of phosphorus-containing minerals in the sediment (*7*) (>50 g kg^{-1} of dry sediment or 5% P) (Fig. 1B). The predominant phosphorus mineral phase was hydroxyapatite [$Ca_5OH(PO_4)_3$], which was determined by x-ray diffraction (XRD) analysis (*7*). Fifty grams of P per kg of sediment is equivalent to 270 g of hydroxyapatite per kg of sediment. Therefore, more than 25% of the solid phase in this layer was hydroxyapatite, which is one of the major

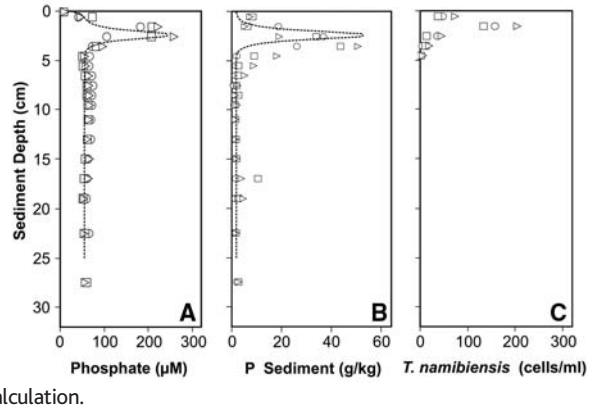

Fig. 1. Sediment profiles from the Namibian shelf (22°10′S, 14°03′E; water depth 70 m). (**A**) Phosphate concentrations in the pore water (μM) at different sediment depths (cm). (**B**) Phosphorus content of dried sediment (g kg^{-1}) at different sediment depths. (**C**) Biomass of *T. namibiensis* (cells ml^{-1}) at different sediment depths. Three parallel measurements are shown as indicated by the different symbols. The dashed lines show the steady-state concentration of pore water phosphate and the amount of phosphorus accumulating as predicted by the model calculation.

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mineral precursors in the formation of phosphorite deposits.

To gain a quantitative understanding of the measured pore water and solid phase-concentration profiles, we used a spreadsheet model similar to that of Schulz (*11*). Diffusive transport of phosphate [diffusion coefficient in sediment $D_{sed} = 4.1 \times 10^{-10}$ m^2 s^{-1} with a temperature of 11°C and a porosity of 0.9 for HPO_4^{2-} as a major species, following thermodynamic calculations (*12*)] was calculated for a one-dimensional (1D) column of 100 cells using an explicit numerical solution of Fick's laws of diffusion (*7*). Boundary conditions were 5 μM phosphate in the bottom water and precipitation of hydroxyapatite when concentrations exceeded 40 μM, which reflects saturation with respect to hydroxyapatite. Thus, the measured pore water profile of Fig. 1A reflects a steady-state situation for production of dissolved phosphate by the bacteria and simultaneous precipitation of hydroxyapatite. Fitting the model to the measured pore water concentration (Fig. 1A, dashed line) resulted in pairs of values; fast phosphate release and fast precipitation, or slow phosphate release and slow precipitation. Laboratory experiments on apatite precipitation as well as the calculation of the necessary diffusive calcium supply for this precipitation confined the range of plausible values for simultaneous release and precipitation of phosphate. As long as a near steady-state condition persisted for ~3 to 14 months, phosphate release rates between 20 (at 3 months) and 6 (at 14 months) nmol liter^{-1} s^{-1} would lead to the observed amounts of precipitated phosphorus in the sediment. (Fig. 1B). The shape of the curve of Fig. 1A, is matched by a phosphate release between 20 and 6 nmol liter^{-1} s^{-1}. Under these circumstances, 3 to 14 months of constant phosphate release would lead to the observed amounts of hydroxyapatite in the sediment (Fig. 1B, dashed line). In contrast, the dissolution of hydroxyapatite after a periodic release of phosphate would be much slower because it is controlled only by diffusion (*7*). *Thiomargarita* cells picked manually and incubated in artificial media in the laboratory (*7*) showed an increase in concentration between 0.011 and 0.028 pmol of phosphate liter^{-1} s^{-1} cell^{-1} with a mean value of 0.018 pmol of phosphate liter^{-1} s^{-1} cell^{-1} (Fig. 2). In comparison, the predicted phosphate release of 6 to 20 nmol of phosphate liter^{-1} s^{-1}, produced by 250 cells ml^{-1} counted in the field, equals an increase in concentration of 0.024 to 0.08 pmol liter^{-1} s^{-1} cell^{-1}. These data confirm that *T. namibiensis* alone could be responsible for the observed pore water phosphate peak and the resulting precipitation of hydroxyapatite.

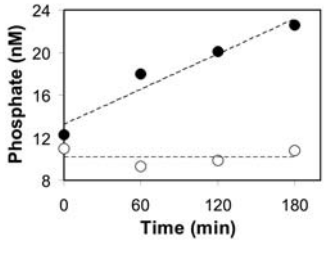

Fig. 2. Phosphate release under anoxic conditions from 50 cells of *T. namibiensis* after 24 hours of anaerobic preincubation, compared to a control (open circles) not containing *T. namibiensis*. Solid circles show mean values of three independent measurements. The single measurements are available in (*7*).

Polyphosphate occurs in nearly all living organisms (*13*), but only some bacteria and yeasts are capable of accumulating large amounts. Bacterial phosphate accumulation has been most thoroughly studied in wastewater treatment plants, where bacteria are used to remove phosphate. To initiate luxury uptake of phosphate by bacteria in a wastewater treatment plant, it is necessary to introduce an anaerobic phase whereby phosphate is released and acetate is taken up and stored, for example, in the form of polyhydroxyalkanoate (PHA). Acetate uptake and storage require energy which, in the absence of an electron acceptor, the bacteria can gain from the breakdown of polyphosphate and consequent release of phosphate. In the aerobic phase that follows, the polyphosphate-accumulating bacteria gain energy by oxidizing the stored carbon using oxygen as the electron acceptor, and they take up an excess of phosphate, which they store as polyphosphate (*14*, *15*). This results in a sludge rich in bacterial polyphosphate, which can be removed from the system.

Based on our incubation experiments, we hypothesize that the mechanisms of phosphate uptake and release in *T. namibiensis* are similar to that of polyphosphate-accumulating bacteria in wastewater, even though their main energy source is considered to be the oxidation of sulfide with nitrate or oxygen as the electron acceptor (*3*, *16*). In vitro, enhanced rates of phosphate release were induced under anaerobic conditions only when acetate was added to the medium. In addition to the many large sulfur globules that were observed in the cells, smaller inclusions were visible in differing amounts (Fig. 3, A and B). Specific staining (*7*) demonstrated that most of the smaller inclusions were polyphosphate (Fig. 3C). The remaining small inclusions did not stain with Nile red, a specific stain for PHA, but were stained dark brown with iodine (*17*) (Fig. 3D) suggesting that they consisted of glycogen or another polyglucose.

T. namibiensis appears to have a life mode that is unusual for marine bacteria. Under anoxic conditions, it takes up sulfide and, presumably, acetate, which appears to be stored as glycogen. Because there is an insufficient supply of a suitable external electron acceptor, internally stored nitrate and polyphosphate are sacrificed and sulfide is oxidized to elemental sulfur to gain energy. Under oxic conditions, the bacterium can gain energy from the oxidation of both sulfur and, presumably, glycogen. At the same time, it invests energy in the accumulation of polyphosphate and nitrate, the latter of which is stored in a central vacuole at concentrations of up to 0.8 M (*3*). Thus, *T. namibiensis* is able to take up each of these chemical compounds under conditions where the chemicals are readily available and use them under different redox conditions, when they are a valuable energy source that would otherwise be impossible to obtain at that time. The observation

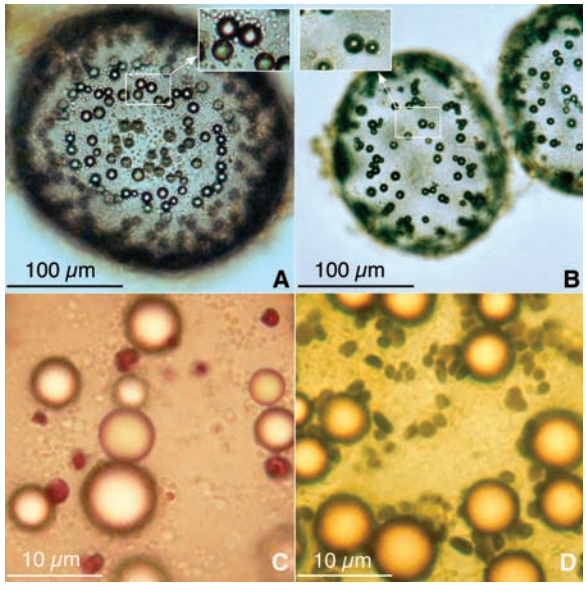

Fig. 3. *T. namibiensis*. (**A**) A single cell of *T. namibiensis* with many smaller inclusions apart from the large sulfur globules. Inset: higher magnification image of the inclusions. (**B**) A single cell of *T. namibiensis* with few smaller inclusions. Inset: higher magnification image of the inclusions. (**C**) Small inclusions stained dark red for polyphosphate with toluidine blue. Many unstained inclusions can be seen. (**D**) Small inclusions stained with iodine, showing a dark brown color typical for glycogen.

that phosphate release could be induced only when acetate was added to the medium shows that the breakdown of polyphosphate is an auxiliary metabolism, which explains why it occurs only episodically and why phosphorus does not continuously accumulate with increased depth.

A connection between polyphosphate-accumulating bacteria and phosphorite formation was proposed two decades ago (*2*, *18–20*). The main arguments in favor of a bacterial involvement were microfossils resembling sulfur bacteria enclosed in phosphorite deposits, for example, in the Miocene Monterey Formation (*18*), and the finding of low C:P ratios in recent *Beggiatoa* mats (*20*). Early diagenetic precipitation of phosphorite minerals has also been reported from the Santa Barbara Basin, where elevated pore water nitrate concentrations after sediment centrifugation suggest an involvement of large sulfur bacteria (*21*). There are also reports of phosphatized bacteria from the Namibian shelf (*22*), which seem to resemble *Thiomargarita*. Because recent phosphorite formation and high biomass of large sulfur bacteria largely occur in the same areas, phosphorite formation through the activity of large sulfur bacteria could be a widespread phenomenon and is likely to also have been important in the past.

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Cardiovascular Risk Factors Emerge After Artificial Selection for Low Aerobic Capacity

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In humans, the strong statistical association between fitness and survival suggests a link between impaired oxygen metabolism and disease. We hypothesized that artificial selection of rats based on low and high intrinsic exercise capacity would yield models that also contrast for disease risk. After 11 generations, rats with low aerobic capacity scored high on cardiovascular risk factors that constitute the metabolic syndrome. The decrease in aerobic capacity was associated with decreases in the amounts of transcription factors required for mitochondrial biogenesis and in the amounts of oxidative enzymes in skeletal muscle. Impairment of mitochondrial function may link reduced fitness to cardiovascular and metabolic disease.

Several investigations link aerobic metabolism to the pathogenesis of cardiovascular disease. Large-scale epidemiological studies of subjects with and without cardiovascular disease demonstrate that low aerobic exercise capacity is a stronger predictor of mortality than other established risk factors (*1–4*). In patients with type 2 diabetes, low aerobic capacity is associated with reduced expression of genes involved in oxidative phosphorylation (*5*). In insulin-resistant elders, there is a 40% reduction in mitochondrial oxidative and phosphorylation activity, largely attributable to impaired skeletal muscle glucose metabolism (*6*). These observations are consistent with impaired regulation of mitochondrial function as an important mechanism for low aerobic capacity and cardiovascular risk factors linked to the metabolic syndrome. These risk factors include weight gain, high blood pressure, reduced endothelial function, hyperinsulinemia, and increased triglyceride concentration in blood. The working hypothesis of the present study was that rats selected on the basis of low versus high intrinsic exercise performance would also differ in maximal oxygen uptake, mitochondrial oxidative pathways, and cardiovascular risk factors linked to the metabolic syndrome.

In previous work, we began large-scale artificial selection for low and high aerobic treadmill-running capacity with the genetically heterogeneous N:NIH stock of rats as the founder population (*7*). Eleven generations of selection produced low-capacity runners (LCRs) and high-capacity runners (HCRs) that differed in running capacity by 347% (Fig. 1A). The founder population had a capacity to run for 355 ± 144 m (23.1 min) until exhausted. On average, the treadmill-running capacity decreased 16 m per generation in LCRs and increased 41 m per generation in HCRs in response to selection. At generation 11, the LCRs averaged 191 ± 70 m (14.3 min), and the HCRs ran for 853 ± 315 m (41.6 min). For this study, we used young adult rats (ages 16 to 24 weeks) derived from generations 10 and 11 to test our hypothesis that risk factors for common dis-

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