Reports

A Thiosulfate Shunt in the Sulfur Cycle of Marine Sediments

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The oxidation of sulfide, generated by bacterial sulfate reduction, is a key process in the biogeochemistry of marine sediments, yet the pathways and oxidants are poorly known. By the use of ^{35}S-tracer studies of the S cycle in marine and freshwater sediments, a novel shunt function of thiosulfate ($S_2O_3^{2-}$) was identified. The $S_2O_3^{2-}$ constituted 68 to 78 percent of the immediate HS^--oxidation products and was concurrently (i) reduced back to HS^-, (ii) oxidized to SO_4^{2-}, and (iii) disproportionated to HS^- + SO_4^{2-}. The small thiosulfate pool is thus involved in a dynamic HS^--$S_2O_3^{2-}$ cycle in anoxic sediments. The disproportionation of thiosulfate may help account for the large difference in isotopic composition ($^{34}S/^{32}S$) of sulfate and sulfides in sediments and sedimentary rocks.

A LARGE AMOUNT OF THE ORGANIC matter that is deposited on the sea floor along continental margins is oxidized through the bacterial reduction of SO_4^{2-} to HS^- (*1*). Recent radiotracer studies of sulfate reduction combined with mass balance calculations have shown that most (up to 95%) of this HS^- is reoxidized to SO_4^{2-} (*2*). Oxygen, NO_3^-, or Fe(III) and Mn(IV) compounds are among the potential oxidants of HS^- in the sediment (*3*). Thiosulfate is an important product of HS^- or pyrite oxidation with O_2 (*4*), but its significance in anoxic environments has been uncertain.

The discovery of thiosulfate disproportionation as an energy metabolism in several strains of sulfate reducing bacteria (*5, 6*) indicated that $S_2O_3^{2-}$ might act as an intermediate in the S cycle. Thiosulfate has two S atoms of which the inner (sulfonate) atom has the same oxidation state, +6, as sulfate, whereas the outer (sulfane) atom has the same oxidation state, −2, as sulfide. The reduction of thiosulfate, for example, by sulfate reducing bacteria, thus involves a valence shift of only the inner atom from +6 to −2

$$S_2O_3^{2-} + CH_3COO^- + H^+ \rightarrow 2HS^- + 2CO_2 + H_2O \quad (1)$$

The oxidation of $S_2O_3^{2-}$ correspondingly involves a shift of the outer atom from −2 to +6

$$S_2O_3^{2-} + 8FeOOH + 14H^+ \rightarrow 2SO_4^{2-} + 8Fe^{2+} + 11H_2O \quad (2)$$

During $S_2O_3^{2-}$ disproportionation, the two atoms do not change valence, although both products, SO_4^{2-} and HS^-, differ from the mean oxidation state (+2) of $S_2O_3^{2-}$

$$S_2O_3^{2-} + H_2O \rightarrow SO_4^{2-} + HS^- + H^+ \quad (3)$$

Thiosulfate disproportionation is a type of inorganic fermentation that does not require an electron acceptor or donor. In order to discriminate the three types of $S_2O_3^{2-}$ conversions, concurrent radiotracer experiments were performed with either the inner or outer S atom labeled with ^{35}S. Because the rapid isotopic exchange reactions between HS^- and several of the reduced S species: S_n^{2-}, S°, and FeS (*7*) it was not possible by use of ^{35}S to identify net transformations between these compounds or to calculate absolute rates of HS^- transformation. All these reduced S species have therefore been combined in the analyses and are designated S_{red}.

In a time-course study of $S_2O_3^{2-}$ transformations in anoxic sediment slurries, the $S_2O_3^{2-}$ was consumed within 2 hours at a rate of 35 μM per hour (Fig. 1). The experiment in which the inner S atom of $S_2O_3^{2-}$ was labeled showed that 33% of the thiosulfate became reduced to HS^-. The experiment in which the outer atom was labeled showed that 10% of the $^{35}S_2O_3^{2-}$ became oxidized to SO_4^{2-}. The remaining 57% was disproportionated to HS^- + SO_4^{2-}. The experiment of Fig. 1 was repeated in sediments from a Danish estuary, a eutrophic lake, and a freshwater stream with similar results.

Studies of the temperature dependence of $^{35}S_2O_3^{2-}$ transformation in this sediment slurry showed that rates reached a maximum in the mesophilic temperature range of 25° to 32°C (*8*). The presence of this maximum strongly suggests that both oxidation and

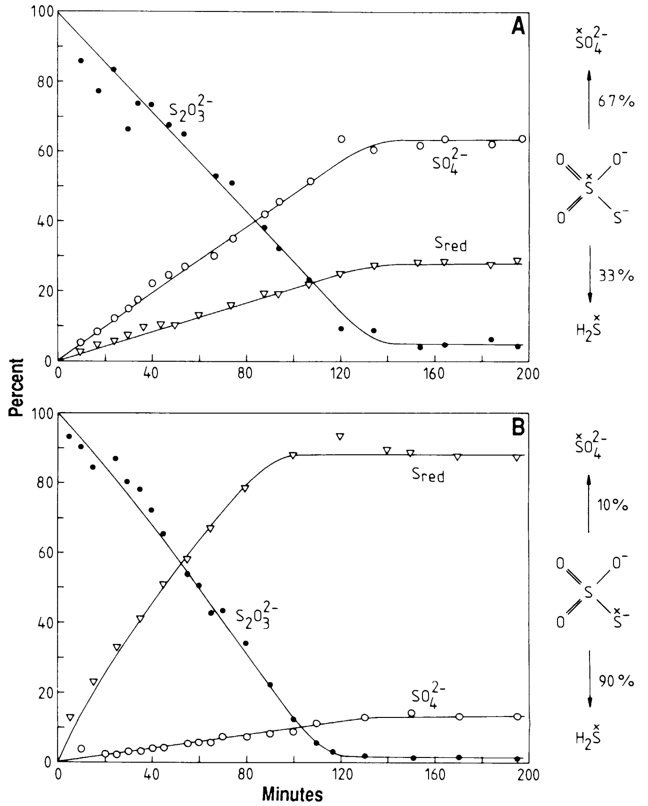

Fig. 1. Transformations of radiolabeled $S_2O_3^{2-}$ into SO_4^{2-} or HS^- (reduced S, S_{red}) in anoxic sediment slurry from the brackish Kysing Fjord, Denmark. The $S_2O_3^{2-}$ was ^{35}S-labeled in either the inner (**A**) or outer sulfur atom (**B**) (*17*). Conversion of the inner (oxidized) S atom to sulfide represents reduction (33%); conversion of the outer (reduced) S atom to sulfate represents oxidation (10%); the remaining $S_2O_3^{2-}$ conversion is due to disproportionation (57%).

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disproportionation of $S_2O_3^{2-}$ were biologically catalyzed processes rather than inorganic reactions.

To further characterize these reactions, I also studied the transformations of SO_4^{2-}, HS^-, and $S_2O_3^{2-}$ in undisturbed cores of sediment from the central Kattegat situated at the transition between the Baltic Sea and the North Sea (Figs. 2 and 3). Molecular O_2 was present to a depth of 9 mm in the sediment, which was brown and oxidized (Eh > 0 mV) down to a depth of 2 cm. Sulfate reduction occurred even in the oxidized zone but peaked at the top of the reducing sediment (Fig. 2A).

Disproportionation, oxidation, and reduction of $S_2O_3^{2-}$ took place concurrently at all depths of the sediment. Disproportionation accounted for 62% of the $S_2O_3^{2-}$ transformation in the uppermost 1 cm but dropped to 35% at a depth of 8 to 10 cm (Fig. 2B). Oxidation reached its peak contribution of 37% at the transition between the oxidizing and reducing sediment zones. Reduction became gradually more significant with depth.

Only about 10% of the HS^- from SO_4^{2-} reduction was permanently trapped as pyrite and other sulfide minerals in the accumulating sediment of central Kattegat (*2*). Free HS^- was not detectable (<1 μM) at depths less than 10 cm; thus, HS^- diffusion through this zone is precluded. The remaining 90% of the HS^- must consequently have been rapidly reoxidized in the sediment microenvironment where it was formed.

Sulfide oxidation was demonstrated throughout the sediment on evidence from labeling with $H^{35}S^-$. The immediate oxidation products of HS^- were 64% $S_2O_3^{2-}$ and 36% SO_4^{2-} in the top 1 cm, which was mostly oxic (Fig. 2C). The contribution of $S_2O_3^{2-}$ increased to 75% in the reducing sediment at 8- to 10-cm depth; $S_2O_3^{2-}$ was thus the main product of sulfide oxidation in all layers. Some of this $S_2O_3^{2-}$ was further oxidized to SO_4^{2-}, while some was reduced back to HS^- (Fig. 2B). Based on the measured distributions of $S_2O_3^{2-}$ reduction, oxidation, and disproportionation (Fig. 2B), the terminal products from oxidized sulfide can be calculated. Near the surface most sulfide was oxidized completely to SO_4^{2-}, but at all depths a significant amount was recycled between HS^- and $S_2O_3^{2-}$ at the same oxidation state as sulfide (Figs. 2D and 3). The further cycling of new and regenerated HS^- will ultimately lead to a complete oxidation of 90% of the sulfide to sulfate.

Similar ^{35}S-tracer experiments were done in whole cores and slurries of two other coastal sediments and in Danish lake and river sediments (*7–9*). The results consistently show that (i) HS^- oxidation takes place mostly below the zone with O_2, (ii) $S_2O_3^{2-}$ is the main (60 to 85%) oxidation product, and (iii) 24 to 62% of the HS^- is recycled by way of $S_2O_3^{2-}$.

Disproportionation of $S_2O_3^{2-}$ was everywhere found to be an important part of the $S_2O_3^{2-}$ transformation, whereas oxidation and reduction varied with depth in the sediments. Thiosulfate-disproportionating bacteria have been enriched or isolated from sediments of both Kysing Fjord and Kattegat, as well as from the Danish Limfjorden and Brabrand Lake (*5*). Population densities in Kattegat reached $>10^6$ cm^{-3} on the basis of viable (MPN) counting techniques (*9*). This was close to the total density of sulfate reducing bacteria, many of which should also be capable of $S_2O_3^{2-}$ disproportionation. Laboratory tests have shown that 8 out of 19 strains of sulfate reducing bacteria can disproportionate $S_2O_3^{2-}$ (*10*).

Concentrations of $S_2O_3^{2-}$ are generally low in the porewater of marine and freshwater sediments [<1 to 10 μM; (*7, 11*)]. Low concentrations are not surprising in consideration of the versatility of bacterial $S_2O_3^{2-}$ metabolism: in the presence of organic substrates, $S_2O_3^{2-}$ can be reduced by most sulfate-reducing bacteria [(*12*) Eq. 1]; in the presence of appropriate electron acceptors, it can be oxidized by lithotrophic S bacteria such as thiobacilli [(*13*) Eq. 2]; in the absence of both organic substrates and electron acceptors it can be disproportionated [(*5, 6*) Eq. 3]. In temporally and spatially heterogeneous sediments, for example, the rhizosphere of salt marshes, conditions seem more favorable for transient accumulations of $S_2O_3^{2-}$ (*14*).

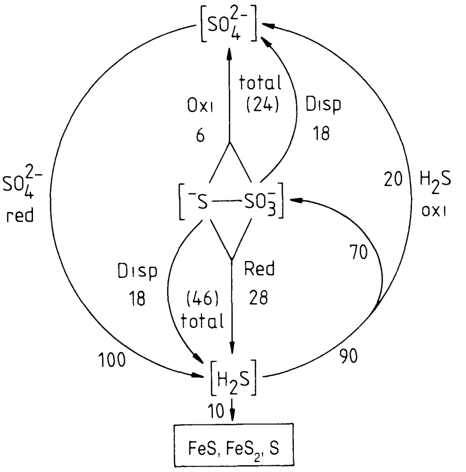

Fig. 3. Summary of the sulfur cycle in anoxic sediment with emphasis on the thiosulfate shunt. Data from depths of 4 to 6 cm in Kattegat were used to calculate processes of reduction (Red.), oxidation (Oxi.), and disproportionation (Disp.). Process rates are expressed as percent of the sulfate reduction rate (for explanation of calculations see text).

On the basis of the present results, I conclude that the "thiosulfate shunt" is important for the coupling of reductive and oxidative pathways of the S cycle (Fig. 3) and thus for the regulation of electron flow in sediments. The flow is shifted toward sulfate in the oxidized surface layers and toward sulfide in deeper layers, but $S_2O_3^{2-}$ is consistently a key intermediate. The HS^--$S_2O_3^{2-}$ cycle is interesting because most of the S atoms do not change valence (−2).

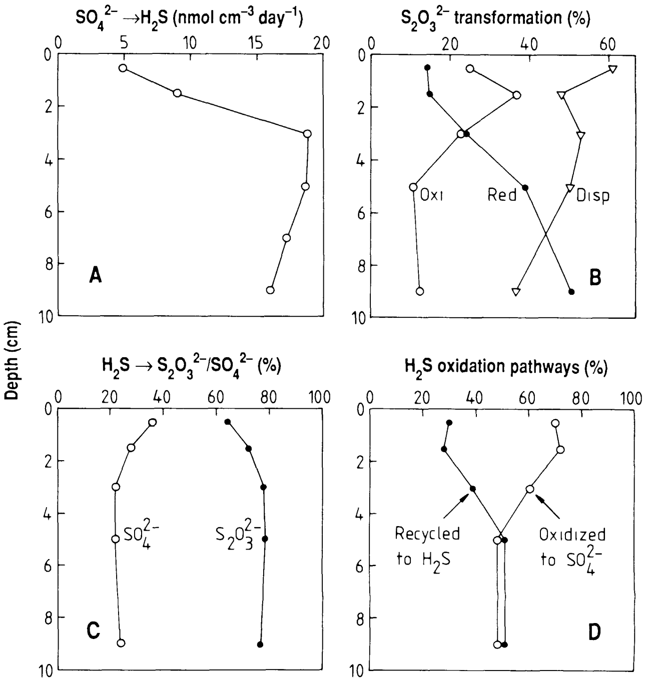

Fig. 2. Depth distributions of ^{35}S-labeled S transformations in undisturbed sediment cores from Kattegat; (**A**) bacterial sulfate reduction; (**B**) oxidation (Oxi.), reduction (Red.), and disproportionation (Disp.) of $S_2O_3^{2-}$ calculated from parallel incubations with inner and outer labeled $S_2O_3^{2-}$. Each process is presented as a percent of the total $S_2O_3^{2-}$ transformation at each depth. (**C**) Oxidized products of HS^-; the $S_2O_3^{2-}$ plus SO_4^{2-} pools accounted for 90 to 100% of the dissolved ^{35}S after precipitation with $ZnCl_2$, indicating that other S oxyanions were not important carriers of radiotracer. (**D**) Calculated pathways of oxidized sulfide after complete transformation of thiosulfate (*19*).

The cycle is therefore partly decoupled from the oxidation of organic matter or the reduction of electron acceptors.

The thiosulfate shunt can help account for the large isotopic fractionation of ^{34}S to ^{32}S between sulfate and sulfides in sediments. It has long been an enigma why the apparent isotopic fractionation during sulfate reduction in marine sediments is commonly between 35 and 60 per mil whereas pure cultures of sulfate reducing bacteria cause a fractionation of only 10 to 30 per mil (*15*). Recycling via thiosulfate will tend to increase the isotopic difference between sulfate and reduced S, mostly because the inner (+6) S atom of thiosulfate is strongly enriched in ^{34}S relative to the outer (−2) S atom (*16*). When the $S_2O_3^{2-}$ is disproportionated, the ^{34}S-rich inner atoms are converted to SO_4^{2-} while the ^{34}S-poor outer atoms are converted to HS^-. The thiosulfate shunt will thereby tend to recycle lighter S in the reduced state and convert heavier S to sulfate.

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17. Results are from a single but representative slurry experiment. The isotopes were added to two separate but identical slurries kept under N_2 at 22°C. The sandy mud had an organic content of 1.8% dry weight. In situ temperature was 4°C and salinity was 15 per mil. The in situ SO_4^{2-} concentration was 13 mM, and $S_2O_3^{2-}$ was below the detection limit by ion chromatography, 10 μM. An unlabeled pool, 75 μM, of $S_2O_3^{2-}$ was therefore added to the two identical slurries. The sediment contained 81 μM HS^- plus 8.2 μmol of FeS and 19.9 μmol of FeS_2 per gram of slurry. Subsamples were taken without air contact, fixed with $ZnCl_2$, and centrifuged. Radiolabeled $S_2O_3^{2-}$ and SO_4^{2-} were separated in the supernatant by ion chromatography and counted. Reduced ^{35}S (S_{red}) comprised HS^-, S_n^{2-}, S°, FeS, and FeS_2 and was all collected by $H_2{}^{35}S$ distillation from washed sediment by the chromium reduction technique (*18*) and counted. Radioactivities are expressed as percent of added $^{35}S_2O_3^{2-}$ radioactivity.
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19. Sediment cores were incubated for 18 hours at the in situ temperature (5.5°C). The sediment was sandy mud from 33-m water depth; organic content was 4% dry weight. The ^{35}S-tracer techniques are described in (*7*, *20*).
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Hind Limbs of Eocene *Basilosaurus*: Evidence of Feet in Whales

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New specimens of middle Eocene *Basilosaurus isis* from Egypt include the first functional pelvic limb and foot bones known in Cetacea. These are important in corroborating the intermediate evolutionary position of archaeocetes between generalized Paleocene land mammals that used hind limbs in locomotion and Oligocene-to-Recent whales that lack functional pelvic limbs. The foot is paraxonic, consistent with derivation from mesonychid Condylarthra. Hind limbs of *Basilosaurus* are interpreted as copulatory guides.

WHALES ARE REMARKABLE among mammals in being fully aquatic, and their transition from land to sea is among the most interesting problems of evolution (*1–3*). Most mammals use limbs, particularly hind limbs, in locomotion. Modern cetaceans live in water and lack hind limbs entirely, retaining only rod-like vestiges of pelvic bones, femora, and rarely tibiae embedded in musculature of the ventral body wall (*4*, *5*). Limbs are important for understanding the early evolution of whales. Hind limb buds have long been known in embryonic cetaceans up to 32-mm crown-rump length (*6*), and adults with externally projecting rudiments are also known (*7*). We now describe evidence of functional hind limbs in a cetacean.

Basilosaurus is a large serpentine Eocene vertebrate discovered early in the 19th century when it was described as a reptile and named "king lizard" (*8*). Richard Owen demonstrated the mammalian characteristics of *Basilosaurus* and, within mammals, its cetacean affinities (*9*). Two species are known: *B. cetoides* from the late Eocene of the southeastern United States and *B. isis* from the late middle Eocene of Egypt (*10*, *11*). The most complete *Basilosaurus* specimens known previously were two partial skeletons of *B. cetoides* collected by C. Schuchert in Alabama in 1894 and 1896 for the U.S. National Museum (USNM). One of these specimens, USNM 12261, includes left and right innominate bones of the pelvis and a partial femur (*12*, *13*); these remains were considered vestigial and functionless (*14*), an interpretation consistent with loss of functional hind limbs in modern whales.

In 1987 and 1989 we mapped 243 partial skeletons of *B. isis* and 77 partial skeletons of smaller archaeocetes (*15*) in the desert of Zeuglodon Valley (ZV), 50 km west of Fayum oasis in north central Egypt (*16*). All occur in a 90-m-thick stratigraphic section of shallow marine sandstones and shales of the Gehannam and Birket Qarun formations of late middle Eocene age (*17*). Excavations in 1989 yielded several nearly complete skeletons combined in the reconstruction shown in Fig. 1A. These indicate that *B. isis* had 7 cervical, 18 thoracic, and 42 lumbar and caudal vertebrae (Fig. 2), 9 more than the number of vertebrae shown in reconstructions of *B. cetoides* (*13*, *14*). Limb and foot bones described here were all found in direct association with articulated skeletons of *B. isis* and undoubtedly represent this species. Specimens are conserved in the Cairo Geological Museum (CGM) and University of Michigan Museum of Paleontology (UM).

The innominate (Fig. 1B) is a straplike coossification of an elongated pubis and a relatively small ilium and ischium, each contributing to a well-defined acetabulum. The pubis and ischium surround a small obturator foramen. Left and right pubes fit togeth-

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