The ecophysiology of sulfur isotope fractionation by sulfate reducing bacteria

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Studies of microbial sulfate reduction have suggested that the magnitude of sulfur isotope fractionation varies as a function of ambient sulfate concentration, with a critical threshold at approximately 200 μM. Sulfur isotope distribution among minerals in Archaean rocks suggested small sulfur isotope fractionations, and has been interpreted as suggesting Archaean sulfate concentrations of less than 200 μM. We have demonstrated that isotope fractionation varies as a function of sulfate concentrations over a much larger range of concentrations.

We examined the concentration-dependence of sulfur isotope fractionation during sulfate reduction using two well-characterized mesophilic sulfate-reducing δ-proteobacteria grown in continuous culture between 0.1 and 6 mM sulfate. The relationship between S-isotopic fractionation and sulfate abundance differed markedly between strains. *Desulfovibrio vulgaris* str. Hildenborough cultures showed a large and relatively constant isotope fractionation ($^{34}\varepsilon_{SO4\text{-}H2S} \cong 25‰$) over the experimental range of sulfate concentrations. Over the same concentration range, and in stark contrast to *D. vulgaris*, fractionation by *Desulfovibrio alaskensis* strain G20 appeared to show a strong correlation with sulfate concentration. Both relationships can be modeled as Michaelis-Menten (MM) relationships between sulfate concentration and fractionation. However the MM constants are very different, suggesting that fractionations respond in dramatically different ways to sulfate concentrations.

Together, these data reveal complexity in the sulfate concentration-fractionation relationship, both at the scale of an individual strain and over a wider range of sulfate concentrations than previously appreciated. This implies that the sulfur isotope fractionation generated during sulfate reduction, and its linkage to environmental sulfate concentrations, is in part a function of strain-specific physiology, potentially related to the affinity of sulfate-reducing microorganisms for both sulfate and electron donors. We present a simple model in which the ratio of MM relationships for sulfate and electron donor uptake produces the relationship seen in experimental studies: a MM relationship with sulfate concentration, and a hyperbolic relationship with growth rate.

Both biological and environmental factors, including strain-specific adaptations to low environmental sulfate concentrations, influence the fractionation recorded in geological samples. We consider the effects of selective pressure on the evolution of sulfate and electron acquisition machinery for over the course of Earth history, complicating efforts to reconstruct ambient sulfate concentrations from sedimentary sulfur isotopic compositions, but perhaps shedding new light on microbial evolution.

References

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Enzyme-specific sulfur isotope fractionation during microbial sulfate reduction

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Abstract

Microbial sulfate reduction (MSR) provides a critical link between Earth's exogenic sulfur, carbon, iron and oxygen cycles. Decades of research suggest that MSR is the prevailing driver of mass-dependent variance in sedimentary sulfur isotope records. MSR couples the oxidation of organic matter to the reduction of sulfate, producing sulfide that is depleted in the heavier isotopes (^{33}S, ^{34}S, ^{36}S) relative to the lightest stable sulfur isotope (^{32}S). This biological signal is captured in modern sedimentary pore-waters and authigenic minerals throughout geologic time. Therefore, precise interpretation of isotopic records requires an understanding of the biochemical controls on sulfur isotope fractionation. Here we provide the first direct measurement of the kinetic isotope fractionation imparted by the keystone MSR enzyme – dissimilatory sulfite reductase (DsrAB). The major and minor enzyme-specific sulfur isotope fractionation factors ($^{34}\varepsilon_{DsrAB}$ and $^{33}\lambda_{DsrAB}$) will be reported and their relevance discussed. Furthermore, the major isotope fractionation ($^{34}\varepsilon_{DsrAB}$) from Bacterial (*Desulfovibrio vulgaris*) DsrAB experiments is consistent at 20 and 31°C, as are experiments performed with DsrAB from a hyperthermophilic Archaeaon (*Archaeoglobus fulgidus*) at 65°C. These values are a mere 30-60% the magnitude of previous indirect $^{34}\varepsilon_{DsrAB}$ fractionation estimates (*1*, *2*), though still explain the bulk of sulfur isotopic fractionations observed in pure culture experiments – also be summarized and discussed here. Further, the DsrAB fractionation describes the lower limit approached by MSR at high metabolic rates in both laboratory experiments and modern marine sediments (~17.3‰)(*3*, *4*). Directly determining individual enzyme induced fractionations is a fundamental step toward establishing a biochemical foundation for interpreting geobiological sulfur isotope records, both modern and ancient. Further, this begins a broader effort to better constrain the biochemical mechanisms underpinning S isotope distributions as a variable function of microbial metabolism(s). This approach has been profound in establishing our understanding for the central role RuBisCO plays in C isotope fractionations and the global C cycle, and is long over due in application to MSR and Earths S cycle. Background readings are listed below. These address putative fractionation capacity of the MSR metabolism as a whole (*5*), as well as historical approaches, pitfalls and successes (*6*), in the on-going effort to untangle the complexities of S chemistry and metabolic isotope fractionation in MSR.

References and Further Reading:

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Sulfur isotope fractionation during microbial sulfate respiration: intracellular influences, environmental controls, and evolutionary responses

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Dissimilatory sulfate reduction is a respiratory process used by some bacteria and archaea to generate energy under anaerobic conditions. Aqueous sulfate serves as the terminal electron acceptor in this process, leading to the oxidation of organic carbon compounds and sometimes hydrogen, and to the production of aqueous sulfide. Dissimilatory sulfate respiration was one of the first microbial metabolisms to be isotopically characterized through culture experiments [1], with ^{32}S-bearing sulfate consumed preferentially to ^{34}S-bearing sulfate. In the intervening half-century, pure culture experiments have played a critical role in characterizing this fractionation. Recent work has precisely calibrated how fractionation magnitude correlates inversely with the average sulfate reduction rate of an individual cell but correlates directly with extracellular sulfate concentrations [2, 3, 4]. Fractionation at the low-rate limit approaches levels defined by thermodynamic equilibrium between aqueous sulfate and sulfide [3], while the fractionation trajectories away from this limit appear to be strain specific.

Here we introduce a quantitative model for sulfur isotope fractionation during microbial sulfate dissimilation that reproduces both of these characteristics. It builds on theoretical [5] and practical [6] demonstrations of metabolic reversibility, and explicitly links fractionation, reaction reversibility, and intracellular metabolite concentrations [7]. It also leads to predictive relationships of fractionation with extracellular sulfate and sulfide concentrations, as well as with intracellular sulfate reduction rates. These relationships are observable characteristics of sulfate-respiring bacteria and archaea in the laboratory and in nature. They are the basis for interpreting fossil S isotope fractionation patterns in the rock record in terms of ancient organisms and their environmental interactions. Both in concept and application, then, sulfur isotope fractionation is a phenotypic trait.

Microbial evolution experiments provide a first step towards evaluating the evolutionary robustness of the sulfur isotope phenotype. We describe a series of laboratory evolution experiments with isogenic sulfate-reducing cultures that were designed to select for increased growth rate. The metabolic fractionation model allows the evolutionary response of the sulfur isotope phenotype in these experiments to be decomposed in its physiological, enzymatic, and environmental parts.

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