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Predictive isotope model connects microbes in culture and nature

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In PNAS, Wing and Halevy (1) present a new model that quantitatively describes the magnitude of sulfur isotope fractionation produced by dissimilatory microbial sulfate reduction (MSR). MSR is a major player in the global biogeochemical cycles and is responsible for the respiration of up to 30% of organic matter in marine sediments (2). This metabolism produces large isotope effects, in which the product, sulfide, is depleted in the heavy isotopes (^{33}S, ^{34}S, and ^{36}S) relative to the most abundant isotope ^{32}S (3), enriching modern seawater sulfate in ^{34}S by about 21‰ (parts per thousand) compared with mantle sulfur. Sedimentary sulfur minerals preserve a record of this effect and are used to track changes in the sulfur isotope composition of seawater and the biogeochemical sulfur, carbon, and oxygen cycles through geologic time (4). Such reconstructions require an understanding of factors that control the magnitude of sulfur isotope effects and dictate the fractionation of sulfur isotopes by sulfate reducers under a range of growth conditions.

All models of sulfur isotope fractionation during MSR, including that of Wing and Halevy (1), attempt to describe the interpretations of sulfur isotope signals produced in inherently complex natural systems and formalize trends bolstered by decades of observations and laboratory studies. Some of the most prominent trends show that: (i) the fractionation of S isotopes correlates inversely with the cell-specific sulfate reduction rates (csSRR) (Fig. 1), implying that the sluggish flow of electrons toward the sulfate-reducing pathway increases the magnitude of isotope fractionation ($^{34}\varepsilon$) (3, 5–8); (ii) the magnitude of isotope fractionation depends on the actual electron transfer pathway and organism (Fig. 1) (9); and (iii) low fractionations are likely in sulfate-limited environments (10, 11).

The new model by Wing and Halevy (1) relies on these observations and interprets some of the model parameters in the light of organismal biochemistry to get around

the microbe- or pathway-specific effects. Conceptual origins of this work date back to the model put forward by Rees (12) in the early 1970s. Rees' model (12) explained the net observed sulfur isotope fractionations by considering the following biochemical steps involved in MSR: activation of sulfate as adenosine-5′-phosphosulfate (APS), reduction of APS to sulfite, and further reduction of sulfite to sulfide. The model assigned intrinsic isotope fractionation factors for the two reduction steps and for the sulfate uptake, as do Wing and Halevy (1), and explored the range of reversibilities at each step. A later study by Farquhar et al. (13) added triple sulfur isotope systematics ($^{32}S/^{33}S/^{34}S$), whereas Brunner and Bernasconi (14) updated the fractionation factors to explain large (>50‰) sulfur isotope fractionations observed in nature. These models could explain the range of sulfur isotope fractionations seen in nature, but their output was not related to environmental parameters, such as the concentrations of sulfate and sulfide, limiting the predictive power. Experimental tests of the assumptions made by these models have also proven difficult.

The Wing and Halevy model (1) is the first to explicitly interpret some of its free parameters using thermodynamics and the influence of electron transfer to the sulfate-reducing pathway. The reversibility is elegantly related to the free energy of reactions and processes (also see ref. 15). The estimated free energy of the reactions under standard conditions (ΔG^0) then allows the reversibility to be quantified as a function of activities of products and reactants. With reasonable assumptions (e.g., fast equilibrium for H_2S inside and outside of the cell), the new model predicts the net fractionation from only three assumed parameters: (i) sulfur isotope effect during the uptake of sulfate (other fractionation factors are fixed), (ii) overall redox potential of the cell, described as the ratios of oxidized and reduced forms of menaquinone, and (iii) a scaling factor, interpreted as the
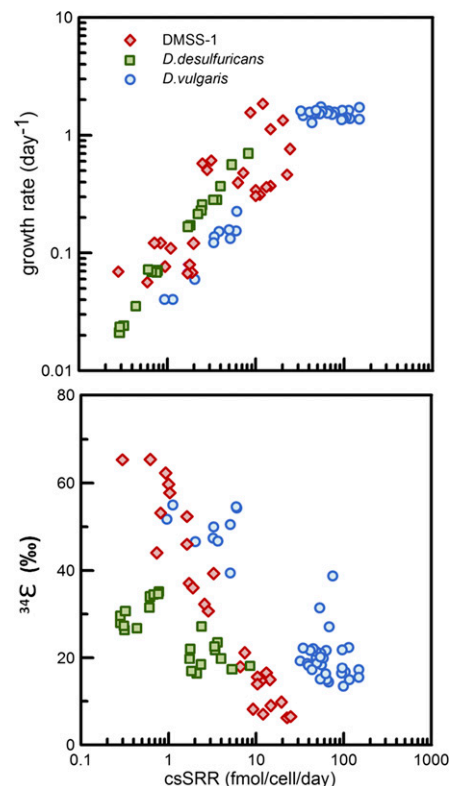

Fig. 1. Relationship between cell specific sulfate reduction rates, growth rates, and isotope fractionation factors for three different strains of sulfate-reducing bacteria. Data from refs. 5–8.

ratio of in vivo enzyme activities to those measured in in vitro crude cell extracts. One of the main contributions of this model will be to inspire future experimental tests of these generalizations.

The Wing and Halevy model (1) uses the results of recent culture studies (5, 6, 8, 13) and produces some new, experimentally testable predictions and observations. As mentioned earlier, experimental studies show a tight correlation between $^{34}\varepsilon$ and cell-specific sulfur reduction rate (csSRR), but the exact relationship differs from one model microbe to another (Fig. 1). Wing and Halevy

(1) attribute this difference to species-specific properties: for example, differences in the activities and abundances of respiratory enzymes, with higher abundances expected at higher growth rates (1). The authors propose proteomic tests for these predictions. Proteomic studies of sulfate reducers are few, but a study by Zhang et al. (16) showed the same abundance of dissimilatory sulfite reductase (Dsr) in cultures of *Desulfovibrio vulgaris* grown on lactate and formate, despite different growth rates. This discrepancy encourages future proteomic analyses of samples from continuous cultures and measurements of Dsr abundances as a function of csSRR, rather than growth rate. Assumptions about enzyme abundances, activities, csSRRs, and growth rates intrinsic to different microbes can also be investigated by mutagenesis experiments, comparative genomics, measurements of the isotopic composition of intracellular inorganic sulfur species, and simple biochemical assays that target the abundances of specific redox-active components, such as ferredoxin, NADH/NAD$^+$, and cytochromes, all recognized players in the electron transfer toward the sulfate-reducing pathway of different sulfate-reducing bacteria (9, 17).

The new model by Wing and Halevy (1) is also used to clarify the relationship between the magnitude of isotope fractionation and environmental sulfate levels, a key question related to the cycling of sulfur on early Earth. Because S isotope fractionations in the rocks of Archean age (>2.5 billion years ago) are typically small, low micromolar levels of seawater sulfate were assumed (10, 11), although alternative explanations also existed (18). Wing and Halevy (1) suggest that fractionations as large as 60–70‰ are possible in the presence of micromolar sulfate, as long as sluggish net respiration rates can be maintained. This theory is consistent with a recent report of high fractionations from a low sulfate environment (10), but experimental verification of the model assumptions will be truly challenging.

Wing and Halevy (1) predict that *D. vulgaris* reducing much less than 1 fmol of sulfate per cell per day should produce $^{34}\varepsilon$ of 60‰. For DMSS-1, a recent marine isolate different from *D. vulgaris*, the

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predicted rate (csSRR) is smaller than 0.5 fmol sulfate per cell per day irrespective of sulfate level (figure 3 in ref. 1). Note that much slower csSSRs, from 0.1 to 0.0001 fmol per cell per day (19), are observed in nature. In lactate-grown cultures, 0.5 fmol per cell per day roughly corresponds to the growth rate of 0.05 per day (Fig. 1). In continuous cultures, this growth rate translates to the turnover time of 20 days, and three-times longer experiments are ideal to ensure a complete reservoir exchange, which is possible but not trivial. Furthermore, the maintenance energy requirements may set the low limit on the respiration rate attainable in chemostat experiments, such that csSRRs much smaller than 0.5 fmol sulfate per cell per day may not be attainable (20). The use of high-energy electron donors, such as glucose (5), may be one way to circumvent the issue of maintenance energy, although such donors are not commonly explored in experimental studies. Therefore, a fundamental value of models, including the one by Wing and Halevy (1), is the ability to make predictions under environmental conditions that elude culture experiments (10, 19).

Microbial sulfate reduction connects ecology, biochemistry, geochemistry, physiology, and Earth history. The predictive power of the model by Wing and Halevy (1) improves our quantitative understanding of these links and provides a new tool with which to explore the evolution of the sulfur cycle from billions of years ago to today.

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