Sulfur Isotope Effects Associated With Microbial Metabolism

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Abstract

Desulfobacterium autotrophicum is a sulfate reducer isolated from marine-shelf sediments. *D. autotrophicum* has the ability to reduce sulfate to sulfide as a way to obtain energy. *D. autotrophicum* is versatile in the number of different types of electron donors and carbon sources that it can utilize.

From 2008-2009, Dr. James Farquhar and collaborators conducted a series of experiments in which *D. autotrophicum* were cultured. During the experiments, temperature and electron donor concentration were manipulated to explore the effect that these variables would have on the magnitude of fractionation between sulfate in the growth medium and excreted sulfide. For my study, the isotopic concentration of the sulfur in the initial sulfate and the sulfide that was excreted as a result of the bacterium's metabolism, was analyzed. These analyses document varying degrees of fractionation that relate to the use of different substrates. Higher magnitude fractionations were observed in experiments that utilized a smaller concentration of the electron donor.

Background

Fractionation observed between the initial sulfate and the excreted sulfide is caused by the multi-step metabolism of *D. autotrophicum.* External sulfate, internal sulfate (sulfate within the cell), APS, sulfite and HS each constitute an individual sulfur pool. A sulfur pool is a discrete reservoir of sulfur with its own isotopic composition. Each step in the reduction process has a fractionation factor associated with it. There is a fractionation of approximately 3‰ associated with the initial uptake of external sulfate into the cell. There is a very small fractionation associated with the transfer of sulfur from internal sulfate to APS and it has been deemed negligible when accounting for the overall fractionation associated with the entire metabolic pathway.

There is a fractionation factor of approximately 25‰ associated with the transfer of sulfur from APS to sulfite and a fractionation factor of approximately 50‰ associated with the transfer of sulfur from sulfite to sulfide (Brunner and Bernasconi, 2005). Each of these steps contributes to the overall fractionation of sulfur isotopes as sulfur flows through the cell. For example, when sulfur in the internal sulfate is reacted with ATP and forms APS, there is a measurable change in the isotope ratios between the sulfur in the sulfate and the sulfur in the APS. Flow through the cell coupled with mixing of the sulfur pools are the key contributors to the fractionation associated with sulfate reducers. Mixing between the various sulfur pools (i.e. reversals in the pathway) leads to changes in the $\Delta^{33}S$ of the sulfur (Farquhar et. al, 2007).

The overall magnitude of fractionation between external sulfate and secreted sulfide is controlled by environmental factors such as temperature, electron donor type, and the availability (concentration) of the electron donor (Brunner & Bernasconi, 2005). These environmental factors impact the magnitude of fractionation by manipulating the flow of sulfur through the cell. It has recently been suggested that factors associated with the manner in which sulfite reductase may also influence fractionations of sulfur (Bradley et al., 2011). Sulfite reductase is a transport enzyme within the cell that is responsible for enabling and catalyzing the multistep process of reduction of sulfite to H_2S.

Methods

Culturing of Bacteria

Each individual culture was primarily artificial seawater with a concentration of 0.8g/2L of $NaSO_4$ as well as the various electron donors: butyrate and $H_2 + CO_2$. Each experiment has a starting sulfate with a known isotopic composition and the excreted sulfide. The first two experiments were cultured with $CO_2 + H_2$, offering concentrations as high as 3bars of pressure and as low as only 10cc of H_2. The second two experiments used a short chain fatty acid, butyrate, commonly found in butter. The two butyrate experiments required concentrations of butyrate at 20mM and 10mM. The experiments used for this project were conducted at a constant 27°C. This project focused on the experiments conducted with 20mM of butyrate as well as the 3bar and 10cc hydrogen experiments because at lease 8 samples have to be analyzed to obtain one value for the fractionation in a single experiment.

Sample Preparation

The sulfate and sulfides that were extracted from the cultures were precipitated as $BaSO_4$ and ZnS, respectively. Then, the barium sulfate was reduced to Ag_2S (acanthite) by reacting the barium sulfate with heat and a Thode solution that is composed of hydrochloric acid, hypophosphorus acid, and hydriodic acid to form H_2S. The H_2S was then precipitated in a silver nitrate solution to form the Ag_2S. The sulfur in the ZnS samples was extracted in a similar procedure but with 5N HCl. The samples were then aged for one week and then rinsed with water and ammonium hydroxide before drying in an oven.

Gas Source Mass Spectrometry

Depending on the amount of silver sulfide per sample available, 2.5~3.0mg of silver sulfide was weighed out and placed into a $1cm^2$ ethanol-cleansed aluminum foil packet. After that, the packet was loaded into a nickel reaction vessel. Once the vessel and the manifold were leak checked to ensure a closed system, the packet was reacted with F_2 for a minimum of 8 hours at ~200°C.

The impurities were extracted and the SF_6 peak was detected using gas chromatography. It was then isolated between two liquid N_2 cooled traps. After that, the SF_6 was fed into the bellows that ultimately pass it to the source. Finally, isotope ratios were determined and converted to $\Delta^{33}S$ and $\delta^{34}S$ values.

Figure 1. Reduction apparatus. For scale, the marked tube is 18cm in length.

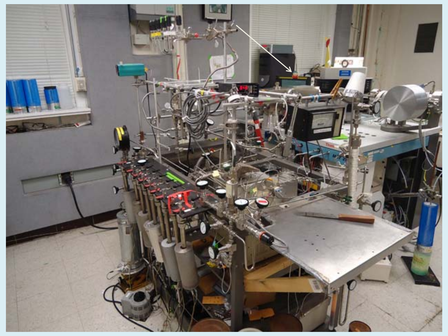

Figure 2. Fluorination line. Scale: Eric Cartman's height: 7.7cm

Figure 3. Thermo Finnegen MAT 253 mass spectrometry. Scale: Cartman's height = 7.7cm

Discussion

Figure 4 displays data from this study as well as data from Johnston et. al (2007). There is a difference of 16.8± 2.6‰ between the experiment using 10cc of hydrogen and the experiment using 3bars of hydrogen. The highest fractionation, approximately 24.3± 0.6 ‰, was observed in the experiment that restricted the electron donor.

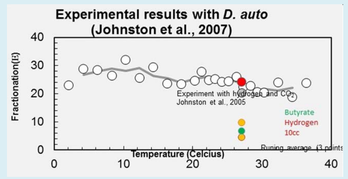

Figure 4. Data Comparison.

Figure 5. Constraints on Fractionation.

Differences between the experiments using hydrogen as the restricted electron donor and the experiments using butyrate yielded a change of fractionation between 2.28± 0.01‰ (restricted H_2 experiments were enriched in ^{34}S). The differences observed in this study represent different magnitudes of fractionation as a result of different growth conditions. Changing substrate and the concentration of the electron donor produced different levels of fractionation.

Figure 5 displays data from the same experiments in a different context. The umbrella shaped field represents constraints on fractionation due to metabolism within the cell (Brunner and Bernasconi, 2005; Farquhar et. al, 2007). These constraints are based on the Brunner and Bernasconi (2005) model of dissimilatory sulfate reduction. Older models were more simplistic but were unable to account for fractionation more negative than -46‰ (Rees, 1973). The purpose of representing the data on this plot is to show that the data fall within the field predicted by this model.

The white data points in figure 3 represent fractionation associated with butyrate (Johnston et. al, 2007). The data collected in this study yield smaller fractionations for the high hydrogen conditions and for the butyrate when compared to those of Johnston et al. (2007) for the similar conditions. The culturing techniques were slightly different in the two experiments which may be a reason for this difference. The qualitative relationship for fractionations between low versus high hydrogen concentration and hydrogen vs. butyrate are still consistent between the two datasets.

Implications

The experiments show a dependence of the magnitude of the fractionation on the availability and type of electron donor. While this is not a new finding, these experiments, and further analyses of other experiments in this set may be useful for understanding the nature of the relationship between electron donors and fractionations which may provide insight into sulfur isotope signatures in Earth's rock record.

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References

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