Connecting bacterial ROS cycling to the production of Mn oxides

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Reactive oxygen species (ROS) can drive the cycling of numerous important elements in the environment. Previously we have shown that the bacterium, *Roseobacter* sp. AzwK-3b, governs the oxidation of Mn(II) via enzymatic production of superoxide. Another ROS, hydrogen peroxide, is also important in this pathway. In fact, in reactions with only Mn(II) and abiotically generated superoxide, we find superoxide alone is not enough to produce Mn(III,IV) oxides. The scavenging of hydrogen peroxide (via the addition of catalase) is required to generate Mn oxides abiotically. Thus, *R*. AzwK-3b must produce superoxide and also scavenge hydrogen peroxide to form Mn oxides. To obtain a greater understanding of biological factors involved in this process, proteins were extracted from *R*. AzwK-3b cultures and examined for their connection to Mn(II) oxidation. Similar to the pathway identified in experiments with *R*. AzwK-3b whole cell cultures and cell-free filtrate, Mn(II) oxidation within soluble protein extracts is initiated by superoxide. Further, an in-gel Mn(II) oxidation assay revealed a protein band that could generate Mn oxides in the presence of soluble Mn(II). This Mn(II)-oxidizing protein band was excised from the gel and the peptides were identified via mass spectrometry. An animal heme peroxidase was the predominant protein found in this band. This protein is homologous to the animal heme peroxidases previously implicated as a Mn(II)-oxidizing enzyme within the *Alphaproteobacteria*, *Erythrobacter* SD-21 and *Aurantimonas manganoxydans* strain SI85-9A1. For *R*. AzwK-3b, the mechanism(s) of Mn(II) oxidation by this animal heme peroxidase is unknown, but may include scavenging of hydrogen peroxide, generation of superoxide, or production an organic ligand involved in Mn(III) complexation. Interestingly, the *Alphaproteobacteria, Ruegeria* sp. TM1040, is also known to generate extracellular superoxide but does not oxidize Mn(II) to Mn oxides. Interestingly, the genome of *Ru*. TM1040 does not contain an animal heme peroxidase, which further suggests an important role for this protein in the formation of Mn oxides.

Enzymatic contraints on the global S cycle: *the fractionation factors of Dsr*

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Coupling quantitative geochemical models with enzyme and whole cell observations informs our interpretation of the environmental histories encoded in the geological S isotope records. Much of this preserved record is driven by the central biochemical machinery of microbial sulfate reducers (MSRs) – linking sulphate reduction to organic matter oxidation. Our present understanding of MSR sulfur isotope fractionation stem from a series of robust cellular-scale (*in vivo*) studies [c.f., 1]. Still, biogeochemical models of the S cycle lack fundamental constraints on the fractionations associated with enzymatic S transformations within the MSR pathway, rendering our best models semi-quantitative. Inspired by the early carbon isotope work on RuBisCO and the influence those studies hold on our understanding of the global carbon cycle, we extend our recent open-system *in vivo* [2] and preliminary pure-enzyme (*in vitro*) experimental work. This study is part of a broader effort to precisely calibrate the S-isotope effects associated with *in vivo* sulphate and *in vitro* sulfite reduction, each underpinned by the MSR enzyme dissimilatory sulfite reductase (Dsr).

To provide fundamental boundary conditions for the network fractionation models of MSR, we performed *in vitro* closed-bottle sulphite reduction experiments with purified DsrAB protein. From these experiments we measure the isotopic composition of all S pools: sulfane and sulfonate moieties in reduction products trithonate and thiosulfate, residual, and initial sulphite. From here we apply a modified Rayleigh model to calculate the enzyme-specific isotope fractionation factors for DsrAB ($^{33}\lambda_{Dsr}$, $^{34}\alpha_{Dsr}$). We repeat this procedure over a range of experimental conditions, including temperature and host species. Isotope and mass balance is conserved in all individual experimental volumes allowing us to directly calculate fractionation factors. Interestingly, the major isotope fractionation factors ($^{34}\alpha_{DsrAB}$) we most often observe are significantly smaller than predicted those from equilibrium estimates, but readily fit with indirect estimates from a seminal study by Jørgensen [3].

[1] Harrison & Thode (1958), *Trans. Faraday Soc*., **54**, 84–92. [2] Leavitt et al. 2013. PNAS, *in press*. [3] Jørgensen (1979) *GCA* **43**, 363-374.

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