Characterization of a novel multicopper oxidase that oxidizes Mn(II)

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Manganese (Mn) oxides are some of the most reactive mineral phases in the environment and control the distribution and bioavailability of a variety of toxic and essential elements. Mn oxide minerals are believed to be formed either directly or indirectly through the activities of microorganisms. The ability to oxidize Mn in several phylogenetically distinct groups of bacteria has been attributed to enzymatic oxidation by a multicopper oxidase (MCO), a family of proteins that utilize multicopper atoms in catalytic sites to oxidize their substrates, typically Fe(II) or phenolic compounds, in one electron reactions. Here we report the expression, purification and partial characterization of a Mn-oxidizing MCO and preliminary efforts to reconcile the one-electron chemistry of MCOs with the two-electron oxidation of Mn(II) to Mn(IV) oxide.

Many attempts have been made to purify the suspected Mn-oxidizing MCO from a variety of different bacteria, including species of *Pseudomonas, Leptothrix, Pedomicrobium* and *Bacillus*. In many species of marine *Bacillus* it is the mature spores that oxidize Mn(II). Oxidation occurs on their exosporium, the outermost layer of the spore, encrusting them with Mn(IV) oxides. Previous studies identified the *mnx* genes, including *mnxG*, a putative multicopper oxidase, as responsible for the two-electron oxidation of Mn(II). Its characterization, however, has been hampered by the difficulty in obtaining purified protein. By purifying active protein from the *mnxDEFG* expression construct, we found that the resulting enzyme is a blue (Abs max 590nm) complex containing MnxE, MnxF, and MnxG proteins. The complex oxidizes both Mn(II) to Mn(III) and Mn(III) to Mn(IV) resulting in the formation of a Mn(IV) oxide mineral. X-ray absorption spectroscopy of the Mn mineral product confirmed its similarity to Mn(IV) oxides generated by whole spores or purified exosporium. With the purification of active Mn oxidase, we will be able to unravel the mechanism of Mn oxidation and broaden our understanding of Mn oxide mineral formation and the bioinorganic capabilities of MCOs.

Inventing the Phanerozoic biological pump - and inducing Snowball Earth

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Export production in the modern oceans is dominated by relatively large/biomineralizing eukaryotic phytoplankton, often accelerated by animal-mediated repackaging in the form of fecal pellets, appendicularian houses and carcasses. Prior to the evolution of animals, and the co-evolutionary radiation of photosynthetic eukaryotes, the biological pump would have worked in a fundamentally different fashion. The transition between these two alternate states appears to have begun in the mid-Neoproterozoic (Tonian/Cryogenian), and achieved a recognizably Phanerozoic condition in the early (but not earliest) Cambrian.

Modern aquatic ecosystems exhibit marked hysteresis between clear-water eukaryote-dominated conditions and an alternative turbid-water cyanobacterial state, typically mediated by suspension-feeding metazoans. In marine shelf environments, the particular ability of sponges to draw down turbidity-inducing DOC, and actively select for larger more export-prone phytoplankton, imparts a first-order control on the biological pump. This in turn presents the circumstances for a more general exploitation of the water column by eumetazoans, especially bilaterians, leading to multi-trophic food-webs, enhanced ventilation and complex feedback effects on nutrient cycling and export.

Biomarker evidence suggests that Proterozoic export was dominated by cyanobacteria. The first quantitatively significant occurrence of eukaryotic steranes appears shortly before the Cryogenian glaciations, along with a novel suite of eukaryotic microfossils. Although there is no direct fossil record of animals at this time, there is a case for recognizing these changes as a co-evolutionary consequence of newly introduced animal activty. Intriguingly, current molecular clock analyses place the first appearance of simple (sponge-grade) animals in this same mid-Neoproterozoic time-frame.

Changes to the mid-Neoproterozoic biological pump are likely to have induced major increases in oceanic alkalinity, leading to a draw-down of atmopheric CO_2 and potentially triggering Cryogenian glaciation [1]. As such, the pronounced evolutionary, biogeochemical and climatic perturbations of the terminal Proterozoic may all be causally linked to the evolutionary appearance of animals.

[1] Tziperman, E., *et al*. 2011. Biologically induced initiation of Neoproterozoic snowball-Earth events. *PNAS* **108**:15091-96.

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