Redox and solution behavior of c-type cytochromes from iron mineral-respiring bacteria

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Mono- and polyheme c-type cytochromes have been linked to ferruginous mineral respiration in a variety of bacteria, such as *Acidithiobacillus*, *Geobacter*, *Shewanella*, and *Acidiphilium*. While excellent genetic and genomic studies have been conduscted, there is a paucity of information regarding the purification, manipulation, and redox behavior of these proteins *in vitro*. Many of these proteins are membrane or cell-surface associated, and preliminary evidence suggests that they can act as multiprotein electron transfer complexes. Our research goal was to identify methods for the effective purification and manipulation of these proteins, in order to facilitate subsequent study. Many redox active proteins are extractable by salt (NaCl, KCl) or mild detergent washing of cells, suggesting a cell surface location. Two examples are the polyheme cytochrome c OmcS from *Geobacter sulfurreducens*, and a 42 kDa cytochrome c from *Acidiphilium cryptum*. Sequential extraction of membrane fractions with incrementally stronger detergents yielded additional redox proteins, and this strategy is useful for targeted purification of particular proetins of interest. Since it is suspected that some of these redox proteins are embedded in a biofilm matrix, experiments were conducted to determine whether this material could be digested away to enable purification. Viscozyme, a mixture of polysaccharidases, was effective at depolymerizing the EPS and reducing problems associated with high levels of polysaccharide contamination. Once extracted out of the cellular and extracellular milleau, some of these protins are extremely difficult to work with, due to hydrophobicity and subsequent aggregation. Some of these issues can be circumvented by use of non-denaturing detergents at low (sub-critical micellar concentrations) concentrations. Finally, our extraction and stabilization methods were applied to the directed proteomic detection of large-mass heme c-containing proteins directly from complex microbial communities, with the hypothesis that these proteins are similar in function to those found in pure cultures of iron reducing or iron oxidizing organisms. In all, our research efforts in protein purification of this interesting class of enzymes has revealed useful information regading their behavior, which is critical for successful studies using voltammetry, Optical Waveguide Lightmode Spectroscopy, and other techniques used to characterize these proteins.

Variations in stable sulfur isotopes in acid sulfate soil materials

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This study represents the first comprehensive investigation into the use of stable sulfur isotopes in acid sulfate soil materials (ASS). The aim of this study was to: 1) examine the isotopic composition of a range of acid sulfate soils, 2) compare stable sulfur isotope ratios in terms of the ASS environmental setting and landuse history, and 3) examine the likely utility of using stable sulfur isotopes to identify the source(s) of sulfate (SO_4^{2-}) contributing to the formation of contemporary ASS materials. The sulfur isotopic composition of the acid volatile sulfur (AVS), chromium reducible sulfur (CRS) and soluble SO_4^{2-} fractions were determined on sulfidic materials from coastal and inland floodplain landscapes.

In mangrove sediments the stable sulfur isotopic ratios of iron sulfides were strongly negative, indicating SO_4^{2-} reduction from an open seawater SO_4^{2-} source. In contrast, positive stable sulfur isotope ratios in other acid sulfate soil materials were indicative of a closed freshwater SO_4^{2-} source. At one location, the Tuckean Swamp, both marine and freshwater sources of SO_4^{2-} exert an influence, which may be related to changes in management practices such as opening flood gates to allow the ingress of tidal water. Stable sulfur isotope data for the inland sites suggest SO_4^{2-} has been incorporated into sulfide precipitates from a range of potential sources. Similarities between the $\delta^{34}S$ of the sulfide and soluble SO_4^{2-} ratios at severely acidified sites indicate the oxidation of sulfides is contributing to the sulfur isotope signature of the SO_4^{2-}. This process may be responsible for supplying a source of SO_4^{2-} which contributes to the seasonal formation of monosulfidic black ooze (MBO). The production of $SO_4^{2-}$from sulfides also resulted in the freshwater overlying one study site having a sulfur isotope signature well outside the range previously given for freshwater SO_4^{2-}.

The results indicate the 'open/closed' concept as often used in stable sulfur isotope studies of sulfidic sediments is problematic in ASS landscapes in eastern Australia. The data also indicates that stable sulfur isotope ratios are a valuable tool to help understand environmental processes occurring in ASS landscapes. The application of stable sulfur isotopes in sediments and ground waters to help discriminate and quantify the contribution the oxidation of ASS is making to the SO_4^{2-} concentration of surface and ground waters during different flow regimes is being examined.