Biochemistry of metal interactions with *Shewanella putrefaciens* cytoplasmic membranes

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Gram-negative bacterial cell walls are chemically diverse, component-rich structures. The inner cytoplasmic membrane contains embedded proteins necessary for molecular and ion transport, cellular respiration, cell division, amongst other essential functions for cell survival and proliferation. Soluble metals produced during dissimilatory metal reduction can penetrate into Gram-negative periplasm and saturate the cytoplasmic membrane [1].

Shewanella putrefaciens CN32 was grown in the presence of 1 mM Mn^{2+}, V^{4+}, and U^{6+} ions under both atmospheric conditions as well as anoxia, and the lipid chemistries of the cell membranes were subsequently characterized. Metal speciation was confirmed through scanning transmission X-ray microscopy (STXM) using near-edge X-ray absorption fine structure (NEXAFS) spectroscopy.

Changes in fatty acyl-chain chemistries across metal treatments were subtle and statistically insignificant, although varied significantly across changes in oxygen status. While the metal-induced variations were subtle, drastic changes are not necessary to invoke variations in phase-state chemistry in prokaryotes. It was observed that gel-to-fluid-gel phase transition temperatures increased significantly in anaerobic environments, suggesting a fluidizing effect of atmospheric exposure, and was confirmed through spectrofluorometric lipid microviscosity quantifcation.

Alternatively, phospholipid liposomes were generated from CN32 cells grown both aerobically and anaerobically. The lipid diffusion in these structures was assessed using a pyrene derivative, which forms excimers when embedded in an ordered environment such as within a membrane. Lipid diffusion was observed to vary depending on metal treatment with 1 mM Mn^{2+}, V^{4+}, and U^{6+}. It is believed that the variations in lateral lipid diffusion are due to cross-linking by the soluble metal cations. Combined with the observation that more thermal energy in these cases is necessary to induce lipid phase-state transition, this suggests that the products of dissimilatory reduction may have more of a role in preserving membrane integrity *in situ* than originally believed.

[1] Beveridge, T.J. (2005). *J. Nucl. Radiochem. Sci.* **6**: 7-10.

Determination of trace elements in seawater by ICP-SFMS after Tm addition and co-precipitation

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We report on two novel procedures for the determination of several trace elements in seawater, including elements characterized by very low abundance (sub-0.1 to 1 ng l^{-1}) in the ocean, such as REEs, Hf, and Th. Our methods are based on the procedure developed recently by Bayon *et al.* [1], and applied successfully to a wide range of geological samples. It involves addition of a Tm spike and pre-concentration using co-precipitation, prior to analysis by inductively coupled plasma-sector field mass spectrometry (ICP-SFMS). The addition of a small amount of Tm to the sample produces a positive Tm anomaly in the resulting REE pattern, which allows calculation of precise trace element concentrations. Two different procedures were investigated during the course of this study: 1) co-precipitation onto iron hydroxides (after addition of a Fe-spike); and 2) magnesium co-precipitation (no spike required).

The validity of the procedures was assessed through a series of co-precipitation experiments, using ultra-diluted solutions of a certified rock standard (BIR-1), and analyses of natural water certified reference materials (CRMs). Results obtained for NASS-5, CASS-4, and SLEW-3 are in agreement with published working values for REEs. A set of proposed values for these CRMs, with uncertainties typically better than 8% (RSD), is also proposed for Hf, Zr, and Th. The advantages and disavantages of using the iron vs magnesium co-precipitation procedure for determining trace element concentrations in seawater will be compared and discussed.

[1] Bayon *et al.* (2009) *Geostand. Geoanal. Res.* **33**, 51–62.