Profiling Mercury Binding Thiol Groups in Natural Organic Matter and Methylating Bacteria Using Fluorescent Spectroscopy

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Recent studies have emphasized the crucial role of organic thiols in controlling the fate of mercury (Hg) species, including highly toxic methylmercury, in both biotic and abiotic systems. Reactions of inorganic Hg species with dissolved organic matter (DOM) result in both reduction of Hg(II) and oxidation of Hg(0), depending on the Hg to DOM ratios. These observations suggest the involvement of two competing mechanisms: reduction of Hg(II) by reduced semiquinone moieties and oxidation of Hg(0) by DOM-thiols via oxidative complexation. Similar phenomena of Hg reduction, oxidation, and surface binding have been observed in methylating bacteria. As a key aspect of studying these processes, a robust and sensitive analytical approach for quantifying the organic thiols present in DOM and bacteria is needed but is not currently available. To that end, we utilized a chemical probe with thiol-specific fluorogenic labeling and systematically evaluated the optimum labeling conditions to ensure high sensitivity (nanomolar levels) and selectivity. Application of our method to measure thiols in *Geobacter sulfurreducens* cells resulted in a value of 0.07 μmoles $g^{-1}$and a value of 3.8 μmoles g^{-1} for a complex soil humic DOM sample. The measured thiol values are in good agreement with those predicted values based on experiments with Hg species. Furthermore, we have developed an HPLC-fluorescence method, with detection based on the described fluorogenic labeling procedure, by which the individual thiols in DOM can be resolved from other components of these complex mixtures, thereby allowing us to characterize the individual thiolated compounds. A clearer understanding of the diversity (or lack thereof) of the major thiols in DOM can be useful for predicting the impact of Hg-DOM complexation, as it pertains to Hg uptake by methylating bacteria, as well as other mechanisms of Hg transformation in the environment.