

Profiling mercury binding functional groups in natural organic matter and methylating bacteria

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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 reduced dissolved organic matter (DOM) results in both reduction of Hg(II) and oxidation of Hg(0), depending on the Hg/DOM ratios, and suggests the involvement of two competing mechanisms – reduction by reduced semiquinones, and oxidation by thiol-induced oxidative complexation. Similar behaviors of reduction, oxidation, and surface binding of Hg species have also been observed on mercury-methylating microorganisms, such as *Geobacter sulfurreducens PCA* and *Desulfovibrio desulfuricans ND132*. 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. However, the analysis of organic thiols is not straightforward, primarily due to their low abundance, susceptibility to chemical and photo-chemical oxidation, and the inherent absence of distinguishing spectroscopic characteristics. To overcome these problems, we have utilized a chemical probe with thiol-specific fluorogenic labeling properties. The probe is commercially available as ThioGlo-1. We systematically evaluated the optimum labeling conditions for model thiols (cysteine and glutathione) with the goal of minimizing alterations to the bacteria and DOM samples, while ensuring high sensitivity (nano-molar levels) and selectivity for organic thiols, even in the presence of potentially interfering compounds (e.g., cystine, amino and carboxyl containing non-thiol compounds). We applied the optimized technique for the measurement of total thiols in washed cells of *Geobacter sulfurreducens PCA* directly in phosphate buffered saline solution and a complex natural organic matter sample, humic acids from the International Humic Substances Society. 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 the other components of these complex mixtures, thereby allowing us to measure the individual thiolated compounds. We demonstrate this method for profiling organic thiols in DOM. 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.