

New Methods for Determining Thiols on Bacteria and Natural Organic Matter in Environmental Systems

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DOE/Office of Science/Biological & Environmental Research

Objective

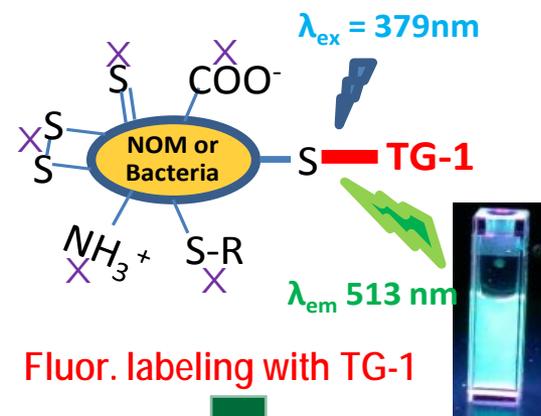
- Develop a new fluorescence-labeling technique to determine total thiols directly on bacterial cells and natural organic matter (NOM) in environmental samples.

New Science

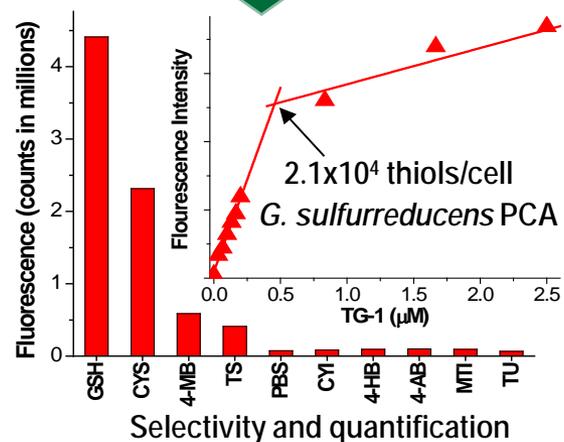
- For the first time, we were able to quantify thiols on intact gram-negative bacterial cells directly in the culture media.
- The method is highly selective and able to quantify thiols on dissolved natural organic matter at sub-micromolar concentration levels.
- New technique enables future studies of the complex interactions and uptake mechanisms of contaminant metals, such as mercury, by bacteria in the environment.

Significance

- Direct quantification of organic thiols on NOM and intact gram-negative bacteria is critically needed for mechanistic understanding of soft metal (e.g., Hg) and biota interactions, metal speciation, and bioavailability.



Fluor. labeling with TG-1



Rao, B., C. Simpson, H. Lin, L. Liang, and B. Gu. 2014. Determination of thiol functional groups on bacteria and natural organic matter in environmental systems. *Talanta* 119:240-247 (doi:10.1016/j.talanta.2013.11.004).

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Organic thiols (R-SH) are known to react and form complexes with some toxic soft metals such as mercury (Hg) in both biotic and abiotic systems. However, a clear understanding of these interactions is currently limited because quantifying thiols in environmental matrices is difficult due to their low abundance, susceptibility to oxidation, and measurement interference by non-thiol compounds in samples. Here, we report a fluorescence-labeling method using a maleimide containing probe, ThioGlo-1 (TG-1), to determine total thiols directly on bacterial cells and natural organic matter (NOM). We systematically evaluated the optimal thiol labeling conditions and interference from organic compounds such as disulfide, methionine, thiourea, and amine, and inorganic ions such as Na^+ , K^+ , Ca^{2+} , Fe^{2+} , Cl^- , SO_4^{2-} , HCO_3^- , and SCN^- , and found that the method is highly sensitive and selective. Only relatively high levels of sulfide (S^{2-}) and sulfite (SO_3^{2-}) significantly interfere with the thiol analysis. The method was successful in determining thiols in a bacterium *Geobacter sulfurreducens* PCA and its mutants in a phosphate buffered saline solution. The measured value of $\sim 2.1 \times 10^4$ thiols cell^{-1} (or ~ 0.07 $\mu\text{mol/g}$ wet cells) is in good agreement with that observed during reactions between Hg and PCA cells. Using the standard addition, we determined the total thiols of two reference NOM samples, the reduced Elliot soil humic acid and Suwanee River NOM, to be 3.6 and 0.7 $\mu\text{mol/g}$, respectively, consistent with those obtained based on their reactions with Hg.

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