Talanta 119 (2014) 240–247

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Determination of thiol functional groups on bacteria and natural organic matter in environmental systems

Balaji Rao [a], Carolyne Simpson [b], Hui Lin [a], Liyuan Liang [a], Baohua Gu [a,*]

[a] Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, United States
[b] University of Tennessee, Knoxville, Department of Chemistry, Knoxville, TN 37996, United States

ARTICLE INFO

Article history:
Received 9 August 2013
Received in revised form
31 October 2013
Accepted 2 November 2013
Available online 9 November 2013

Keywords:
Thiols
Mercury
Fluorescence spectroscopy
Titration
NOM

ABSTRACT

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 μmol g^{-1} 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 μmol g^{-1}, respectively, consistent with those obtained based on their reactions with Hg.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Organic thiols (R–SH) are a class of reduced sulfur compounds that occur in soil, fresh and marine water systems. They are typically found in association with organic materials in the natural environment [1–4]. Thiols (e.g., cysteine and glutathione) of biological origin are known to participate in reactions such as oxidative stress mitigation, metal uptake and detoxification, and bio-molecular activation of microorganisms [5,6]. Thiols along with other reactive functional groups (e.g., carboxylates, quinone and semiquinones, etc.) can govern the fate and speciation of certain metal ions in the environment [4,7,8]. For instance, reactions of inorganic mercury (Hg) species with natural organic matter (NOM) result in both reduction of Hg(II) and oxidation of Hg(0), depending on the redox state and Hg/NOM ratios [7,8]. These studies suggest the involvement of two competing mechanisms: reduction by semiquinones and complexation by thiol-induced oxidation. Similar behaviors of reduction, oxidation, and surface binding of Hg have been observed on Hg-methylating bacteria including *Geobacter sulfurreducens* and *Desulfovibrio desulfurricans* ND132 [9,10]. However, despite their importance, techniques for direct quantification of thiols on bacterial cells and NOM are not readily available [11]. As a key aspect of studying the processes that regulate the fate and transport of such metal ions as Hg, a robust and sensitive analytical approach for quantifying the organic thiols on NOM and bacteria is needed.

Current techniques for thiol measurements include electrochemistry, mass spectroscopy, X-ray absorption spectroscopy (XAS), and chemical derivatization for light based spectroscopic analysis [12–16]. However, direct analysis of thiols in environmental and biological matrices faces significant challenges due to their low abundance, susceptibility to chemical and photochemical oxidation, and inherent absence of distinguishable spectral characteristics. The XAS techniques suffer from low sensitivity with thiol detection limits on the order of micromolar (μM) to millimolar (mM) levels [14]. Although electrochemical techniques are reported to detect nanomolar levels of pure thiol compounds [12,17], they are yet to be demonstrated for direct analysis of thiols on NOM and bacterial cells. Recently, chemical labeling agents have been used to selectively react with thiols to enhance their light absorption or fluorescence emission resulting in sensitivity up to femtomolar levels [16,18]. However, these analyses are often

* Corresponding author. Tel.: +1 865 574 7286.
E-mail address: gub1@ornl.gov (B. Gu).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.11.004

performed in laboratory prepared solutions and involve considerable sample preparation, chemical separation and detection by high performance liquid chromatography [19], thus making them unsuitable for direct quantification of thiols on intact bacteria in the culture media. Furthermore, they usually require a prior knowledge of the nature of thiols in the samples, which presents a problem for measurement of samples such as NOM where information regarding the specific type of thiols is unknown [20].

A different approach to minimize sample alteration has been recently reported for the measurement of unknown thiols using a fluorescence reagent, monobromo(trimethylammonio)-bimane (qBBr), that belongs to a class of bimane compounds [11]. This technique was applied to measure thiols on a Gram positive bacterium, *Bacillus subtilis*, and natural water sample containing NOM. The thiol concentration was quantified by performing fluorescence titration of the sample with incremental amounts of qBBr at levels below and above the total thiols present. The method utilized deionized water as the clean background solution for sample processing and analysis to minimize matrix interferences [11], but the effectiveness of this method in complex matrices, such as phosphate buffered saline (PBS) solutions that are needed to maintain the structure and viability of bacteria cells, was not evaluated. As the authors noted, Gram-negative bacteria, such as *G. sulfurreducens* and *Shewanella oneidensis*, are prone to lysis in deionized water because they have softer cell envelopes than *B. subtilis* [11]. Currently, no effective methodologies are available to quantify reactive thiols on these organisms, which have limited our ability to assess their reaction processes that affect the transformation and transport of metal ions like Hg.

Here, we present an improved measurement technique for thiols in complex environmental matrices using a fluorescence labeling reagent that consists of a thiol-reactive maleimide group, known as ThioGlo-1 (TG-1) (3H-Naphthol[2,1–b]pyran-s-carboxylic acid) [21,22]. TG-1 reacts with R–SH compounds quantitatively through Michael addition mechanism (1:1 on a molar basis). TG-1 has been used for determining thiols in biological proteins and tissues [21,23] but never used for direct quantification of thiols on bacteria and NOM. TG-1 is very sensitive to thiol-containing compounds and exhibits high fluorescence quantum yield after reaction with thiols [22,24]. We report systematic evaluation of the labeling conditions and optimization to minimize potential interferences for thiol determination with a high selectivity and sensitivity. We applied the optimized conditions for the measurement of low micromolar concentrations of thiols on a Gram negative methylating bacterium *G. sulfurreducens* PCA and its mutants in the culture solutions, as well as on two NOM samples.

2. Experimental

2.1. Bacteria and NOM sample preparation

Details of the preparation of bacterial samples have been reported elsewhere [9,10]. In brief, *Geobacter sulfurreducens* PCA and two of its mutant strains were harvested from the growth media in the late exponential growth phase, pelletized by centrifugation (1500*g* for 10 min) in an anaerobic chamber, and the supernatant then discarded. The pelletized cells were re-suspended and washed 3 times in de-aerated PBS solution consisting of 0.14 M sodium chloride (NaCl), 3 mM potassium chloride (KCl), 10 mM disodium phosphate (Na_2HPO_4), and 2 mM potassium phosphate (KH_2PO_4) with pH adjusted to 6.6. The PBS was chosen to mimic the background matrix used for bacterial Hg methylation studies [9,10] and to maintain the structure and viability of bacteria cells. The washed cell suspension was immediately used for TG-1 labeling and analysis. A portion of the suspended bacteria was used for cell number density measurement by analyzing the optical density (OD) at 600 nm, which was further validated by direct cell counting using a hemocytometer under a microscope [9,10]. Thiol measurements were then performed at different cell densities (from 8×10^{12} to 3×10^{13} cells L^{-1}) using both the wild-type (WT) and the mutant strains in PBS. The cell number densities before labeling and after fluorescence measurement were mostly identical, suggesting that no significant cell lysis occurred during analysis.

Two NOM samples, Elliot soil humic acid (HA) and Suwannee River NOM (SR-NOM), were obtained from the International Humic Substance Society (IHSS). These NOM samples were chemically reduced with hydrogen (H_2) in the presence of palladium (Pd) catalyst (5% Pd on alumina powder, 1 g L^{-1}) and stored under anoxic conditions, as described previously [7]. The NOM stock solutions (1 g L^{-1}) were diluted to a final concentration of 50 and 100 mg L^{-1} for HA and SR-NOM, respectively, in PBS before analysis.

2.2. Chemical reagents and standards

TG-1 with a purity > 99.0% was obtained from EMD Millipore chemicals (San Diego, CA) and used without further purification. A stock solution was prepared by directly dissolving the salt in 100% dimethyl sulfoxide (DMSO) and stored at 4 °C. TG-1 working standards (25, 50 or 100 μM) were prepared in acetonitrile from the stock on the day of sample analysis. Stock solutions (10 mM) of organic thiols and non-thiols were prepared in deionized Milli-Q water (> 18 MΩ cm). They include L-cysteine (CYS; > 99%) from Acros Organics, glutathione (GSH) from Fisher BioReagent, thiosalicylic acid (TS; 97%), 4-mercaptobenzoic acid (4-MB; > 99%), L-cystine (CYI; > 98%), 4-aminobenzoic acid (4-AB; 99%), 4-hydoxybenzoic acids (4-HB; > 99%), thiourea (TU; > 99%), and L-methionine (MTI; > 98%) from Sigma-Aldrich.

To study the effects of common cations in the thiol measurement, the stock solutions of iron (Fe^{2+}) (18 mM) and calcium (Ca^{2+}) (25 mM) were prepared in deionized water from ferrous sulfate ($FeSO_4 \cdot 7H_2O$, 99.5%, Avantor Performance Materials) and calcium chloride (anhydrous $CaCl_2$, 96%, EM Science), respectively. Copper (Cu^{2+}) stock solution (16 mM) was prepared from a reference standard (Baker Analyzed Reagent, > 99.9% purity). A metal chelating agent EDTA (ethylenediaminetetraacetic acid potassium salt, Reagent grade) was used to evaluate its ability to mitigate the effect of metal ions during analysis. Potential interference of sulfur-containing compounds was determined in the presence of sulfide (Na_2S, anhydrous salt, 98%, Sigma-Aldrich), thiocyanate (KSCN, 99%, EM Science), 9,10-anthraquinone-2,6-disulfonic acid (AQDS, 98%, Sigma-Aldrich), and sulfite (Na_2SO_3, > 98%, ACS reagent). These compounds were freshly prepared in PBS at 10 mM. Sulfite appeared unstable in PBS so that 1 mM EDTA was added to ensure its stability [25].

2.3. Fluorescence spectroscopic measurement

All fluorescence labeling experiments with TG-1 was performed in dark by mixing 1–3 mL of the sample with 0–0.05 mL of the TG-1 working standards at varying concentrations. Unless otherwise specified, all titrations, including studies of the effect of interfering ionic species, were performed in the PBS at pH 6.6 (used for bacteria samples). The buffer was found to be sufficient in maintaining its pH during all titrations. Reference thiols, bacteria, and NOM samples were allowed to react with TG-1 for ~2 h at room temperature, and this reaction time was sufficient to obtain stable fluorescence emission for all samples. Fluorescence spectra were recorded on a Fluorolog fluorescence spectrophotometer equipped with both excitation and emission monochrometers (Johin-Yvon SPEX Instruments,

Download English Version:

https://daneshyari.com/en/article/7681090

Download Persian Version:

https://daneshyari.com/article/7681090

Daneshyari.com