Dissolved Low-Molecular Weight Thiol Concentrations from the U.S. GEOTRACES North Atlantic Ocean Zonal Transect

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Abstract

Low-molecular weight thiols, including cysteine and glutathione, are biomolecules involved in a variety of metabolic pathways and act as important antioxidant and metal buffering agents. In this last capacity, they represent a potential mechanism for modulating the bioavailability and biogeochemistry of many trace elements in the ocean, particularly for chalcophilic elements (e.g., Cu, Zn, Cd, Ag and Hg). For this reason, and in the context of the international GEOTRACES program that seeks to understand the biogeochemistry of trace elements in the ocean, we measured the concentration of individual dissolved low-molecular weight thiols during the U.S. GEOTRACES North Atlantic Zonal Transect (USGNAZT). Only two thiols were identified, cysteine and glutathione, in contrast to results from the northeast subarctic Pacific Ocean, where the dipeptides glycine-cysteine and arginine-cysteine were also present and γ -glutamylcysteine was dominant. Concentrations of cysteine and glutathione in the North Atlantic Ocean were lower than in the Pacific and ranged from below detection (~ 0.01 nM) to 0.61 nM of cysteine and up to 1.0 nM of glutathione, with cysteine generally more abundant than glutathione. Vertical profiles of cysteine and glutathione were broadly consistent with their biological production, being more abundant in surface water and usually below detection at

depths greater than about 200 m. Subsurface concentration maxima, often co-incident with the deep chlorophyll maximum, were frequently observed but not universal. We conclude that cysteine and glutathione do not make up significant portions of complexation capacity for Cu and Zn in the upper open ocean but could be important for Cd, Hg, and potentially other chalcophiles. Extremely low concentrations of cysteine and glutathione in deep water suggest that higher molecular-weight thiols are a more important ligand class for chalcophiles in that portion of the ocean.

1. Introduction

Thiols (organic compounds possessing -SH functional groups) are molecules synthesized by cells to defend against oxidative stress induced by the presence of reactive oxygen species (Ercal *et al.*, 2001; Rijstenbil, 2002; Sunda *et al.*, 2002). Thiols may also decrease the toxicity of metals by chelating them and thereby lowering intracellular free metal concentrations which agrees with the observed increase in intracellular thiol concentrations in phytoplankton, plants, and fungi as a result of elevated metal exposure (Ahner *et al.*, 2002; Rijstenbil and Gerringa, 2002; Courbot *et al.*, 2004; Dupont and Ahner, 2005). More recently, the role of thiols as metal transporters has received increased attention because many chalcophilic metals possess both micronutrient and toxic properties for cells (e.g., Satoh *et al.*, 2002; Schaefer and Morel, 2009; Semeniuk *et al.*, 2015).

Dissolved thiols are found in measureable concentrations in lake, estuary, and ocean waters. Dissolved thiol concentrations are below 10 nM in open-ocean seawater but have been observed to be greater than 10 nM in regions of high productivity (Tang *et al.*, 2000; Al-Farawati and van den Berg, 2001; Laglera and van den Berg, 2003; Dupont *et al.*, 2006). However, thiol

concentrations greater than 500 nM have been observed in regions where permanently sulfidic conditions are present, such as the Black Sea (Mopper and Kieber, 1991) and sediment pore waters (Chapman *et al.*, 2009).

Many different biogenic thiol compounds are present in marine environments. Compound-specific analyses of dissolved thiols have identified cysteine, glutathione, and γ -glutamylcysteine as the most common low-molecular weight thiols in the natural waters studied (Mopper and Kieber, 1991; Tang *et al.*, 2000; Dupont *et al.*, 2006; Hu *et al.*, 2006). Other thiols, that are less frequently observed in seawater, include the dipeptides cysteinyl-arginine and cysteinyl-glutamine in the North Pacific (Dupont *et al.*, 2006) and phytochelatins, which are oligomers of glutathione, in coastal waters (Ahner *et al.*, 1997; Tang *et al.*, 2000). Cysteine-rich small proteins, such as metallothioneins, are also commonly produced by algal cells (Ahner and Morel, 1995), but dissolved metallothioneins have yet to be detected in the ocean. The diversity of thiols present in natural waters is likely greater than that already described, because unidentified thiol compounds have been observed in chromatograms (e.g., Tang *et al.*, 2000).

Thiol composition and concentration differences among regions of the ocean may be the result of differences in plankton community, their metal exposures, and local environmental factors that influence thiol fate in the water column (Ahner *et al.*, 2002; Dupont and Ahner, 2005). Relevant to GEOTRACES is the implication that changes in plankton community composition could affect chalcophilic metal biogeochemistry by controlling the biogenic thiol assemblage that is produced. As the primary source of thiols is biogenic production, co-variation between dissolved thiols and chlorophyll *a* (Chl *a*) concentrations might be expected and indeed has been observed in the surface coastal ocean (Al-Farawati and van den Berg, 2001). However, thiol concentrations were unrelated to Chl *a* in surface water from both the northwest Pacific and

northwest Atlantic (Dupont *et al.*, 2006; Kading, 2013), which suggests that the sinks (primarily photo-oxidation and biological uptake) for thiols may be just as influential as biogenic production in determining ambient concentrations, especially in oligotrophic surface waters and at depths below the photic zone. For example, photochemical destruction of the thiol pool in the surface ocean has been demonstrated to be on the order of hours (Flock *et al.*, 1997; Winterbourn and Metodiewa, 1999; Laglera and van den Berg, 2006; Moingt *et al.*, 2010). Photochemical decomposition in surface water is consistent with the observation of an increase in thiol concentration from the sea surface to a maximum at the deep chlorophyll maximum, where primary productivity is still active but light levels are lower (Dupont *et al.*, 2006), and may even imply that the time of day could influence vertical distributions of thiols. The decrease is also consistent with biological uptake as cysteine has been observed to be taken up in varying degrees by SAR11 and other α -proteobacteria, which are thought to be the dominant heterotrophic bacteria in the ocean (Kiene *et al.*, 1999; Tripp *et al.*, 2008). Thus, there are both physical and biological controls on the sources and sinks of dissolved low-molecular weight thiols in the ocean, and the controls are likely to interact in complex ways, giving rise to a variety of vertical and horizontal profiles that could obscure correlations with tracers of productivity, such as Chl *a*.

Dissolved thiols have the potential to exist in both reduced (RS^-) and oxidized ($RSSR$) forms, which impacts their ability to complex metals. In addition to a relatively slow reaction rate with molecular oxygen in oxic waters, oxidation of low-molecular weight thiols has been observed to be catalyzed by Cu(II), superoxide, H_2O_2 and ultraviolet radiation (Winterbourn and Metodiewa, 1999; Rigo *et al.*, 2004; Moingt *et al.*, 2010). The presence of Hg and Ag at concentrations comparable to those of thiols has been demonstrated to greatly reduce the rate of glutathione oxidation presumably by strong complexation (Hsu-Kim, 2007). However, this is a situation

unlikely to occur in nature as Hg and Ag are typically 100–1000× less abundant than glutathione. A single measurement suggests that about half the dissolved thiols in the surface ocean are reduced, with the remainder as oxidized dithiol (Dupont *et al.*, 2006).

In an effort to better understand the fates of chalcophilic metals in the ocean and the biogeochemistry of their ligands, we used seawater collected during the U.S. GEOTRACES North Atlantic Zonal Transect (USGNAZT) to quantify compound-specific concentrations of as many low-molecular weight thiols as we could identify and detect. While this kind of study has been completed in the past (e.g., Dupont *et al.*, 2006), we believe this to be the largest such report of species-specific thiol concentrations in the ocean and the first to span an entire basin.

2. Methods

2.1 Cruise track and sampling

The cruise track for the U.S. GEOTRACES North Atlantic Zonal Transect (USGNAZT), also referred to as GEOTRACES Atlantic 03 (GA03), is shown in Figure 1, and consisted of two legs, both aboard the R/V *Knorr*. The first (KN199-4), beginning on 15 October 2010 from Lisbon, Portugal occupied stations in the eastern North Atlantic stretching mostly meridionally south to the Cape Verde Islands, where the leg was cut short by a mechanical problem on 4 November 2010. This leg included stations 9–12, which constituted a short zonal section through a region of higher productivity associated with the Mauritanian Upwelling. An additional component of Leg 1 were surface-only collections from an underway towed “fish” system (Bruland *et al.*, 2005) made during transit from Cape Verde to Charleston, South Carolina from 8

to 26 November 2010 as the ship returned to the U.S. for repair. Leg 2 (KN204-1A & B), from 2 November to 11 December 2011, occupied stations between Woods Hole, U.S. and Cape Verde. This leg featured several closely spaced stations near the western continental margin, station BATS (Bermuda Atlantic Time Series), and the TAG hydrothermal vent.

At each station, water samples for analysis of dissolved thiols were obtained from Niskin bottles deployed from a rosette used to collect water for isotopic and non-contamination prone trace elements analysis (the “ODF Rosette”). The rosette was outfitted with an on-board CTD and triggered manually as electrochemical and depth data were received through a conducting cable. On deck, water was filtered from the Niskin bottles by members of the scientific crew through Acropak filters (0.45 μm , polyethersulfone) into low-density polyethylene bottles (60 mL) that had been previously cleaned with HCl and rinsed with reagent-grade water (resistivity > 18 M Ω -cm). We refer to thiols in these samples as being “dissolved” but they should more accurately be described as “filtered” because colloids and particles less than 0.45 μm could have passed the filter. Samples were frozen (−20 °C) immediately, without further amendment, and kept frozen until processing in the laboratory at WHOI. In this study, we made no effort to preserve the oxidation state of the thiols, but rather make use of a method that reduces all thiols prior to analysis. Therefore, the concentration of thiols present in seawater that is available to participate in metal complexation will be some fraction of the value reported here. There has been no work in seawater, to our knowledge, regarding the effects of freezing on the long-term stability of low-molecular weight thiols. Thus, there is a possibility that some of our analytes were oxidized beyond the formation of disulfide bonds (which our method addresses) to the formation of sulfonates during storage. Such work should be performed to aid in further validating our data.

2.2 Derivitization

Before analysis, frozen seawater samples were thawed overnight in a refrigerator and acidified to 15 mM with methanesulfonic acid (MSA). Samples (50 mL) were derivatized and analyzed according to the methods of Tang and colleagues (2003) with a few modifications (Kading, 2013). Changes to the Tang protocol included use of *tris*(2-carboxyethyl)phosphine (TCEP) as the reducing agent, and addition of the reducing agent after neutralization, rather than before. Prior to derivitization, the pH of the samples was adjusted to between 4 and 5 with 6 M NaOH and the thiols present in the sample were reduced by reaction with TCEP (83 μ L of a 5 mg/mL solution per 50 mL sample) at room temperature for 30 minutes. This results in an in-sample concentration of TCEP of about 33.2 μ M, which results in a TCEP/thiol ratio around 10,000. Samples were then buffered carefully to a pH of ~8.5 for optimal reaction kinetics by addition of 2 M boric acid, 0.8 M NaOH, and 10 mM EDTA solution. Thiol derivitization was accomplished by the addition of 80 μ L of 10 mM monobromobimane (mBBr) which was allowed to react for 2 hours at 60 °C. This recipe results in a nominal TCEP to mBBr ratio of about 2, well below the reductant/mBBr ratio limits suggested by Tang and colleagues (<5.2). We did detailed optimization experiments in seawater from Martha's Vineyard Sound (relatively high thiol and Cu concentrations) and found the ratio of 0.8 to be optimal, but the higher ratio gave better results for open ocean waters like those reported here. The reaction was terminated (and the sample pH adjusted for column retention) with the addition of 800 μ L of 6 M MSA. Standards for calibration were prepared through addition of cysteine and glutathione to an "average" seawater made by combining excess sample into 50 mL volumes that were then derivatized and analyzed in the same way as samples. Similar to Tang et al. (2003), the 50 mL

samples were passed through Waters Oasis HLV 3 cc (60 mg) cartridges at less than 2 mL/min and eluted with 1 mL of methanol into 2-mL amber vials. Methanol eluent was evaporated from the vials overnight with air before derivatized thiols were dissolved in 1 mL of 15 mM MSA for analysis.

2.3 Analysis

Every standard and sample was analyzed with an Agilent 1200 series high performance liquid chromatograph (HPLC). Sample aliquots of 100 μ L were injected onto a Waters Spherisorb 5 μ m ODS2 4.6 $\times$ 250 mm C18 column with attached 4.6 $\times$ 10 mm guard column at 30 $^{\circ}$ C. The elution gradient started at 8% acetonitrile (mobile phase) and 92% water with 0.1% trifluoroacetic acid (TFA) at a flow rate of 1 mL/minute. The percentage of acetonitrile was increased as follows: 8–10% from 1 to 13 minutes; 10–14% from 13 to 20 minutes; 14–16% from 20 to 24 minutes, 16–18% from 24 to 28 minutes, 18–24% from 28 to 35 minutes; 24–80% from 35 to 43 minutes, and from 80% back down to 8% from 43 to 47 minutes, followed by 15 minutes at 8% acetonitrile before the next sample was injected. The flow rate was increased to 1.3 mL/min from 20 to 24 minutes and brought back down to 1 mL/min from 24 to 28 minutes to wash out the reagent peak (Tang *et al.*, 2003). The individual derivitization products were detected by fluorescence (Agilent FLD) with excitation and emission wavelengths of 394 and 470 nm, respectively. This excitation/emission pair was deemed optimal after examining several wavelength pairs and identifying the region of highest signal strength. Detection limits for cysteine and glutathione were about 0.01 nM, but varied based on derivitization efficiency and blanks each day. A typical chromatogram is shown in Figure 2.

We determined retention times for cysteine, glutathione, γ -glutamylcysteine, arginylcysteine, glycylcysteine, N-acetyl-cysteine, homocysteine, and phytochelatins 2-5 (PC2-5; noted in Figure 2) with standards, but identified only cysteine and glutathione in seawater samples. However, there were several chromatogram peaks in samples that were not seen in blanks or corresponded to thiols for which we had standards. The cumulative integrated area for these unidentified peaks was greater than that of glutathione and cysteine, suggesting that a large fraction of the total thiol concentration and metal complexing capacity in these waters was associated with compounds that have not been characterized. The mBBr tag does not exclusively react with reduced thiols, and has been observed to react with other functional groups as well (e.g., Fahey *et al.*, 1980), albeit at lower efficiencies than with thiols. Thus, some of the unidentified peaks could be non-thiol-containing compounds as well as thiols.

Sixteen of the samples were run in duplicate to assess the reproducibility of this technique. These duplicates had average relative standard deviations for cysteine and glutathione concentrations of 34% and 43%, respectively. In addition, an in-house seawater reference, made from pooled extra water, was analyzed 9 times over the course of this work, and these analyses had relative standard deviations of 28% and 31% for cysteine and glutathione analyses, respectively. Thus, a conservative estimate of error might be $\pm 40\%$.

3. Results

Vertical profiles of cysteine and glutathione concentrations are shown in Figures 3–7. The maximum concentrations of cysteine and glutathione observed over the entire sampling campaign were 0.61 nM and 2.21 nM, respectively. The maximum measured cysteine

concentration is lower than the ~2 nM maximum observed in the northeast Pacific Ocean (Dupont *et al.*, 2006), while the maximum glutathione concentration is similar between both basins. At most stations, either surface or near-surface maxima decrease to concentrations below detection at depths of 200–500 m below the surface.

The general range of concentrations observed along the sections agrees reasonably well with expectations from prior studies. For example, Keil and Kirchman (1999) measured the concentration of dissolved free amino acids (DFAA) in the northwest Atlantic Ocean in an oceanographic setting comparable to our stations 2011-6 and 2011-8. They found DFAA concentrations less than 10 nM and with a similar vertical profile to that we observed for cysteine and glutathione. As cysteine typically represents 0.5–2% by mole of amino acids in proteins (e.g., Miseta and Csutora, 2000), we would therefore predict that cysteine concentrations in Keil and Kirchman’s DFAA pool would have been <0.2 nM, which is within the range measured in this study.

Two general profile types were observed for cysteine and glutathione in the North Atlantic Ocean. The majority of the profiles displayed sub-surface maxima of cysteine and glutathione concentrations (e.g., station 2010-10). For most of the profiles of this type, there was also a sub-surface maxima for chlorophyll, but the thiol maxima were generally shallower than the peak in Chl *a*. The remaining profiles showed little evidence of a subsurface peak, and instead had maximum thiol concentrations at the surface. Surface maxima were observed almost exclusively at the nearshore stations (stations 2010-9 through 2010-11; 2011-1 through 2011-6) and were associated with Chl *a* maxima that were broad and near the surface. These results suggest that cysteine and glutathione were covariant, but thiol concentrations were not correlated with Chl *a* ($r^2 = 0.07$ for cysteine, $r^2 = 0.06$ for glutathione). This general trend of broad co-variation was

usually seen for both cysteine and glutathione, but at some stations (2010-1, 2010-7 and 2010-11) subsurface maxima were observed for cysteine, but not glutathione. Across the whole data set, cysteine and glutathione concentrations were not well correlated with each other ($r = 0.58$), but when observed on a station by station basis, about half the stations had $r > 0.7$ between cysteine and glutathione. The stations that had the greatest correlation coefficients between cysteine and glutathione were not systematically distributed, and unrelated to either location, local time of collection, or Chl *a* distributions.

Figure 8 shows the concentration of cysteine and glutathione in surface waters (<20 m) from both the 2010 and 2011 legs, including *ad hoc* surface water sampling from Cape Verde to Charleston, South Carolina between the two official legs of the cruise. Concentrations of both cysteine and glutathione were notably greater near the margins, as might be expected due to the higher levels of primary productivity associated with these waters compared to the gyre. The cysteine to glutathione ratio was near unity and fairly uniform across most of the basin. However, the cysteine to glutathione ratio increased on the margins and was greater on the western than eastern margin. The differences of cysteine to glutathione ratios may reflect differences in plankton community composition, differences in the oxidative stresses experienced by plankton, or perhaps differences in the photo-reactivity of these two compounds.

There is evidence from other studies for photodegradation of thiols (Moffett, 1995; Dupont *et al.*, 2004; Laglera and van den Berg, 2006), which suggests that there is a potential for a sampling time-of-day bias on the observed thiol concentrations for samples from the photic zone, which if strong enough might either present false trends or hide real trends across the basin

depending on the time of day of the sampling. We examined our shallowest samples for any evidence of bias by plotting their concentrations as a function of local sampling time (Figure 9). Some of the samples collected at mid-day had a slightly lower concentration than those collected at other times, but no clear trend was observed (i.e., no significant correlation with hours before/since noon). Thus, it would appear that photodegradation of cysteine and glutathione is less important than other sources of variability in determining the distribution of these compounds in surface seawater.

The TAG hydrothermal field station was the only location where a measurable concentration of either cysteine or glutathione was observed at a depth below 500 m. At that station, a single sample contained a measurable concentration of cysteine from a depth where a plume of hydrothermally impacted water was known to exist (e.g., Fitzsimmons *et al.*, 2015; Hatta *et al.*, 2015; Jenkins *et al.*, 2015; Lam *et al.*, 2015; Measures *et al.*, 2015; Sedwick *et al.*, 2015). Interestingly, this particular plume was not enriched in Cu (Jacquot and Moffett, 2015), and the cysteine enrichment was confined to a single depth at the core of the plume, while other parameters were enriched at several depths above and below the single point for cysteine. Submarine hydrothermal vent plumes are known to be enriched in reduced sulfur, but usually as sulfides. However, Reeves and colleagues (2014) noted that hydrothermal fluids can be highly enriched in methanethiol, but did not report anomalously high concentrations of either cysteine or glutathione.

4. Discussion

4.1 Importance for Cu, Zn and Cd speciation

Concentrations of cysteine and glutathione in the North Atlantic Ocean are relatively low when compared to the concentration of chalcophile ligands, as determined through competitive copper ligand titrations along this section (Jacquot and Moffett, 2015) or in general (e.g., Bruland and Lohan, 2004; Buck and Bruland, 2005; Bundy *et al.*, 2013; Heller and Croot, 2015). This suggests that the low-molecular weight thiols determined in this study are only a small fraction of the whole potential complexation capacity for Cu and, likely, most other chalcophiles as well. Though the low-molecular weight thiols might not be a large fraction of the Cu ligand pool, they might be important if their binding strengths are high. There is some controversy here as IUPAC values for cysteine and the so-called L1 Cu ligand class appear to be similar in this regard when allowances are made for the differences of thermodynamic and conditional data (Berthon, 1995), while more recently Walsh and Ahner (2013) have suggested that the K values (and the K' values implied by them) compiled by Berthon (1995) are substantially too large, offering K' values closer to $10^{11.3} \text{ M}^{-1}$ for Cu(I)-cysteine and $10^{11.9} \text{ M}^{-1}$ for Cu(I)-glutathione. Given the sub-nanomolar concentrations of both thiols observed in this study, the three orders of magnitude difference in K' values controls whether cysteine and glutathione complexes are a dominant Cu species in the open ocean or not. If the more recent and lower K' values are more accurate, then we must come to the same conclusion as Walsh and Ahner (2013): low-molecular weight thiols do not have an important role in Cu complexation in the dissolved phase of the open ocean. The case of Zn mirrors that of Cu as well, with Walsh and Ahner's (2013) K' values substantially less than those determined from titrations (e.g., Ellwood and Van den Berg, 2000; Lohan *et al.*, 2005), leading to the same conclusion that the observed concentrations of cysteine and glutathione have little influence on Zn speciation.

The importance of LMW thiols for Cd may be quite similar to that of Cu. Bruland (1992) documented Cd ligand concentrations in the open North Pacific Ocean that were around 0.1 nM and confined to the upper 200 m, in much the same way that we observed cysteine and glutathione distributed vertically in the North Atlantic Ocean. Furthermore, the K' for this ligand class with Cd was measured to be around $10^{12.0} \text{ M}^{-1}$ (Bruland, 1992), which is quite similar to values reported for 1:1 Cd complexes with cysteine, again, accounting for activity differences (Berthon, 1995). However, Walsh and Ahner (2013) also noted major discrepancies between their results and that of IUPAC with regard to Cd ligands, and this is supported by the work of others as well (e.g., Chekmeneva *et al.*, 2011; Baars *et al.*, 2014). Thus, cysteine and glutathione are likely too weak to make up a significant fraction of Cd speciation in the dissolved phase of the open ocean, similar to Cu and Zn.

Prominence in speciation may not be the final word on the importance of LMW thiols and the biogeochemistry of Cu, Zn and Cd. For example, Semeniuk and colleagues (2015) recently conducted radio-copper uptake experiments from seawater by phytoplankton and bacteria in the presence of natural Cu-complexing ligands as well as in seawater amended with naturally occurring and synthetic ligands. They observed dramatically higher rates of Cu uptake when either cysteine, glutathione, or another “weak” ligand (bathocuproinedisulfonic acid) was added, even though Cu complexes with these ligands should make up only a tiny fraction of total Cu. Thus, while measured equilibrium constants discourage the view that cysteine and glutathione account for a significant fraction of dissolved Cu complexation in the ocean, these low-molecular weight “weak” ligands may have an important role in the marine biogeochemistry of Cu. The same might be true as well for Cu photochemistry, where the tiny pool of cysteine-bound Cu could represent an important control on redox cycling for that metal (Leal and Van den Berg,

1998). Weak ligand facilitation of uptake has also been suggested for Zn (Aristilde *et al.*, 2012) and Fe (Croot and Heller, 2012).

4.2 The Importance for Hg and Ag speciation

Organic ligand concentrations and affinities for Hg have been measured in open ocean seawater in only one case of which we are aware (Lamborg *et al.*, 2004). In that instance, ligand concentrations were sub-nanomolar and conditional affinities were $\sim 10^{23} \text{ M}^{-1}$, which is similar to tabulated values for Hg complexation with cysteine (Berthon, 1995), with conditional corrections. The ranges of stability constants in the IUPAC compilation for Hg with cysteine and glutathione are very large, however, so the most that can be said is that our field data is consistent with the hypothesis that low-molecular weight thiols dominate Hg speciation. Refined laboratory data on thiol affinities for Hg are needed to further test this view.

One of our interests in determining thiol concentrations in open-ocean seawater was to test whether these ligands could exert any control on bioavailability that might modulate Hg(II) conversion into monomethylmercury (CH_3Hg^+) in the ocean, as has been observed during incubations of cultured methylating and non-methylating bacteria (Schaefer and Morel, 2009; Szczuka *et al.*, 2015). As noted, cysteine and glutathione concentrations reach their maxima in waters shallower than 200 m, and often near the surface, which is generally not the case for vertical distributions of CH_3Hg^+ and certainly not on these sections of the North Atlantic Ocean (Bowman *et al.*, 2015). While the lack of correlation between vertical distributions of cysteine, glutathione, and CH_3Hg^+ distributions does not mean that these low-molecular weight thiols play no role in open-ocean Hg methylation, it is clear that, at present, the distribution of the thiols offers no help in predicting the distribution of CH_3Hg^+ .

There is not enough data to assess how important thiols like cysteine and glutathione might be for complexation of Ag in the open ocean. Strong complexation by organic ligands is known to occur for Ag, as Ndung'u and colleagues demonstrated that UV digestion of water samples was necessary to liberate the Ag prior to further analysis (Ndung'u *et al.*, 2006). However, no ligand titrations for Ag have been performed in seawater to our knowledge, preventing comparison of our data to ambient ligand properties.

4.3 First-order modeling of thiol distributions

The general shape of cysteine and glutathione vertical profiles would appear to be consistent with a model consisting of production at either the surface or chlorophyll maximum by phytoplankton, and then dispersion downward and loss to either decomposition or biological uptake. However, it is interesting that measurable concentrations of cysteine and glutathione were observed substantially below the surface and chlorophyll maximum, which begs the question of how these profiles could arise and be sustained. In this section, we developed a simple model to test whether this view is a plausible explanation of thiol distributions in the North Atlantic. The downward dispersion of dissolved thiols could take two forms, either vertical eddy-driven diffusion or particle sinking coupled to remineralization. Thus as a first step and to simplify further modeling, we examined whether particle sinking or vertical diffusion could be determined to be more important in the case of thiols. The downward flux of dissolved thiols associated with vertical diffusion can be modeled as:

$$F_{diff} = -K_z \frac{\partial C}{\partial z}$$

where F is the flux (mole $\text{m}^{-2} \text{d}^{-1}$), K_z is the vertical eddy diffusivity ($\text{m}^2 \text{d}^{-1}$), C is the concentration of dissolved thiol (mole m^{-3}) and z is depth (m). Typical K_z values are on the order of $10^{-5} - 10^{-2} \text{m}^2 \text{s}^{-1}$ (i.e., 1–1000 $\text{m}^2 \text{d}^{-1}$) in this region of the ocean (Ledwell *et al.*, 1993). Because the vertical gradient in thiol concentrations near the surface is on the order of 1 nmole m^{-4} , the model suggests a range of possible values for F_{diff} of 1–1000 nmole $\text{m}^{-2} \text{d}^{-1}$.

The downward flux of thiols associated with particle sinking (ignoring rates of remineralization for the moment to provide an upper end estimate) can be modeled as:

$$F_{sink} = wC_p$$

where w is the vertical sinking rate (m d^{-1}) and C_p is the concentration of particulate thiols (mole m^{-3}). Values of w have been observed to range widely from about 20 to over 1000 m d^{-1} (Trull *et al.*, 2008; Armstrong *et al.*, 2009; McDonnell and Buesseler, 2010). We did not measure the concentration of C_p , but predict that C_p represents about 3% of cysteine and 45% of glutathione amounts in the water, assuming the solid-dissolved phase distribution of thiols is similar to that observed by Dupont *et al.* (2006). Because dissolved thiol concentrations near the surface were 0.1–1 nM, a range for sinking fluxes of particulate thiols of $10^{-2} - 20 \text{nmole m}^{-2} \text{d}^{-1}$ seems reasonable. Thus, to at least a first order estimate, mixing/diffusion would appear to be responsible for most of the vertical dispersion of dissolved cysteine and glutathione.

If eddy diffusion is indeed the primary way in which thiols are moved vertically in the ocean, then concentration profiles could be modeled by the following equation:

$$C_z = C_0 e^{-\left(\lambda/K\right)^{1/2} z}$$

395

396 Where C_z is the concentration (mole m^{-3}) at depth z (m), C_0 is the concentration at the surface
397 (mole m^{-3}), λ is the decay/uptake constant (d^{-1}), and K ($\text{m}^2 \text{d}^{-1}$) is the vertical eddy diffusivity
398 (Glover *et al.*, 2011). The lifetime of thiols ($1/\lambda$) with respect to decay/uptake would therefore be
399 equal to:

400

$$\frac{1}{\lambda} = \frac{z_{5\%}^2}{8.97K}$$

401

402 where $z_{5\%}$ is the depth at which thiol concentrations decrease to 5% of their
403 surface/maximum concentration. Given the vertical eddy diffusivities (K) noted above and the
404 observation that $z_{5\%}$ is about 100 m, the lifetime of thiols suggested by the vertical profiles are on
405 the order of a few days to a century, with the higher end apparently more reasonable based on
406 direct measurements of K at these depths (Ledwell *et al.*, 1993). Lifetimes of highly bioactive
407 compounds like cysteine and glutathione in excess of a few days are not likely, which suggests
408 that the simple model of production at the surface/chlorophyll max and diffusion downward is
409 not appropriate and sources of the thiols below the surface and/or chlorophyll max are needed.
410 Thus, cysteine and glutathione are probably being released and then either destroyed or taken
411 back up again throughout the upper 500 m of the ocean by heterotrophs, and not just by
412 phototrophs in the photic zone. This may be a partial explanation for why poor correlations were
413 observed between cysteine, glutathione, and Chl *a* concentrations, overall. Interestingly, the
414 absence of measurable dissolved cysteine and glutathione below about 500 m suggests that either
415 heterotrophic microorganisms at these depths do not release these low-molecular weight thiols in
416 substantial amounts or that release rates are less than decay by some process. This could be the

result of either the relatively low abundance of heterotrophic microorganisms at such depths, or their relatively low metabolic activity.

One potential source of cysteine, other than exudation from primary producers, is the breakdown of dissolved polypeptides. Keil and Kirchman (1999) reported concentrations of dissolved combined amino acids (DCAA) in the northwest Atlantic Ocean ranging from 167 to 810 nM at a site comparable to our station 2011-4 with regard to proximity to the shelf. Because cysteine typically represents 0.5–2% by mole of amino acids in typical biological tissue (e.g. Miseta and Csutora, 2000), we would predict that the concentration of cysteine in the DCAA pool might represent 0.8 to 16 nM. These predicted concentrations of cysteine are greater than those we measured in the North Atlantic but suggest that protein-associated cysteine is a potentially significant source of upon peptide degradation. Because this potential source of cysteine is 10–100× more abundant than dissolved free cysteine, the breakdown of DCAAs could explain cysteine concentrations in the ocean, as long as DCAAs degrade at a rate that is no more than ~100× slower than the cysteine uptake/degradation rate. Indeed, Keil and Kirchman (1999) found that protein turnover times were, on average, about 30× longer than free amino acids (like cysteine), which suggests that DCAA is a viable source of the dissolved cysteine we observed.

Glutathione is perhaps a bit more difficult to explain if its primary parent material is phytochelatins. In the open ocean, phytochelatins have been observed at concentrations well below that of glutathione (Ahner *et al.*, 1998). This implies that either phytochelatin lifetimes are short in the upper 500 m of the ocean or that release from cells is the primary source of glutathione.

To be sure, the primitive modeling we have presented here is not intended to be a definitive explanation for cysteine and glutathione distributions, but rather suggest some avenues for future

research. It does appear from this initial approach that we can say that a model with only production at either the surface or chlorophyll maximum is not adequate to explain the vertical distribution of these thiols in the North Atlantic Ocean.

5. Conclusions

Our measurements of species-specific thiol concentrations during the U.S. GEOTRACES North Atlantic zonal transect revealed that the thiol composition of the North Atlantic Ocean appears to be quite different from that in the northeast Pacific Ocean, with only two low-molecular weight thiols (cysteine and glutathione) being identified and detected. The vertical profiles and surface horizontal gradient of both thiols exhibited near surface and coastal maxima, often co-varying but not correlating with Chl *a*, which is consistent with biological production by phototrophs. However, the depth of thiol penetration down to 200–500 m suggests that production at the surface is likely not the only source of these compounds, with either release by heterotrophs or degradation of proteins and peptides within the mesopelagic zone the most likely additional sources. The concentrations of cysteine and glutathione observed in the North Atlantic Ocean discourage the view that they represent a significant fraction of complexing ligand classes for Cu and Zn, but are possibly important for less abundant chalcophiles such as Cd and Hg.

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Figure legends

Figure 1. Sampling stations for the 2010 and 2011 legs of the US GEOTRACES North Atlantic
Zonal Transect.

Figure 2. A typical chromatogram showing the retention times for some of the thiol species for
which we had standards.

Figure 3. Vertical profiles of cysteine (open triangles), glutathione (closed circles), oxygen
(black line) and Chlorophyll a fluorescence (gray line) at the stations occupied during the
meridional component of the 2010 leg (from Lisbon to Cape Verde).

Figure 4. Vertical profiles of cysteine (open triangles), glutathione (closed circles), oxygen
(black line) and Chlorophyll a fluorescence (gray line) at the stations occupied along the zonal
Mauritanian Upwelling component of the 2010 leg.

669

670 Figure 5. Vertical profiles of cysteine (open triangles), glutathione (closed circles), oxygen
671 (black line) and Chlorophyll a fluorescence (gray line) at the stations occupied along the zonal
672 western shelf break component of the 2011 leg (Woods Hole to Cape Verde).

673

674 Figure 6. Vertical profiles of cysteine (open triangles), glutathione (closed circles), oxygen
675 (black line) and Chlorophyll a fluorescence (gray line) at the stations occupied along the zonal
676 western North Atlantic basin component of the 2011 leg, including the Bermuda Atlantic Time
677 Series (BATS) station (station 10).

678

679 Figure 7. Vertical profiles of cysteine (open triangles), glutathione (closed circles), oxygen
680 (black line) and Chlorophyll a fluorescence (gray line) at the stations occupied along the zonal
681 eastern North Atlantic basin component of the 2011 leg, including the Mid-Atlantic Ridge TAG
682 site (station 16).

683

684 Figure 8. Surface water concentrations of cysteine and glutathione during the 2010 and 2011 legs
685 of the US GEOTRACES North Atlantic Zonal Transect. Concentrations for both species were
686 highest near the margins.

687

688 Figure 9. Temporal variation of surface water thiol concentrations as a function of the local time
689 when the samples were collected. The symbols are triangles for cysteine, squares for
690 glutathione, open for daylight collections, closed for night.

691

Figure 3 of 9

Figure 4 of 9

Figure 7 of 9

Figure 8 of 9