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Assessment of the potential for copper limitation of ammonia oxidation by Archaea in a dynamic estuary

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ABSTRACT

The distribution and speciation of copper (Cu) in Hood Canal, a fjord in western Puget Sound, Washington, were studied over a 2-year period. Cu is required as a cofactor for many enzymatic pathways, including ammonia oxidation. In Hood Canal, ammonia oxidation is largely dominated by ammonia-oxidizing archaea (AOA), which have high Cu requirements for other processes as well. Dissolved Cu was slightly depleted in the upper water column, and concentrations were almost unchanged from measurements made in the late 1970s, ranging from 4.08 to 6.12 nM. Although this implies that the biological demand is small relative to the large and relatively constant inventory of dissolved Cu, and that Cu limitation is therefore unlikely to influence rates of biological processes, speciation measurements indicated that dissolved Cu is strongly complexed by organic ligands. As a result, bioavailable Cu^{2+} concentrations were considerably lower, varying from 6.14×10^{-15} M to 1.36×10^{-12} M. This is a range that encompasses the threshold ($<\sim 2 \times 10^{-13}$ M) for Cu limitation of ammonia oxidation by *Nitrosopumilus maritimus* SCM1, a representative AOA, in culture (Amin et al., 2013). Furthermore, Cu^{2+} displayed a clear trend over most sampling periods, with Cu^{2+} concentrations one to two orders of magnitude higher below 20 m in the deeper, saline waters, and exhibiting minima in the upper 15 m. The major freshwater input to Hood Canal is not an important source of ligands, which suggests that the ligands are likely produced biologically in the water column and have slow turnover times. In general, ammonia oxidation rates varied considerably but were lowest in the upper water column where Cu^{2+} concentrations were also lowest. Thus, these findings will facilitate further work to ascertain the relative importance of Cu bioavailability in limiting ammonia oxidation rates versus light inhibition, which has frequently been invoked to account for low rates of nitrification in the upper water column.

1. Introduction

There is considerable interest in the speciation of copper (Cu) in estuaries, arising from its toxicity to marine organisms (Buck and Bruland, 2005; Moffett et al., 1997). More recently there has been interest in Cu limitation of various enzymatic processes that are important in the carbon and nitrogen cycles (Jacquot et al., 2013; Maldonado et al., 2006; Peers et al., 2005). Cu limitation can arise when dissolved Cu is tightly complexed by natural organic ligands and concentrations of the free, hydrated cupric ion fraction, Cu^{2+}, are sufficiently low (Moffett et al., 2012). Possibly the most Cu-dependent members of the marine microbial community are the ammonia-oxidizing archaea (AOA; Walker et al., 2010). A genomic survey of the recently isolated ammonia-oxidizing archaeon *Nitrosopumilus maritimus* SCM1 (hereon called SCM1; Könneke et al., 2005) suggested a high reliance on Cu for many aspects of its basic physiology (Walker et al., 2010). In addition to encoding the Cu-dependent metalloenzyme ammonia monooxygenase (AMO), which catalyzes the oxidation of ammonia (NH_3) to hydroxylamine in the first step of nitrification (Vajrala et al., 2013), the SCM1 genome encodes numerous multi-Cu oxidases and blue Cu proteins resembling sulfo- and plastocyanin enzymes that are associated with the electron transport system (Walker et al., 2010). Genes encoding similar blue Cu proteins have been discovered in other AOA genomes (Blainey et al., 2011; Hallam et al., 2006), suggesting that a high demand for Cu may be more widespread among AOA than previously thought. This

Abbreviations: Cu, copper; NH_3, ammonia; AOA, ammonia-oxidizing archaea; AOB, ammonia-oxidizing bacteria; Fe, iron; PNM, primary nitrite maximum; ETSP, eastern tropical South Pacific; O_2, dissolved oxygen; NO_3^-, nitrate; NO_2^-, nitrite; NH_4^+, ammonium; PO_4^{3-}, phosphate; SiO_4^{4-}, silicate; CTD, conductivity, temperature, depth; ID-ICP-MS, isotope dilution inductively coupled plasma mass spectrometry; *L*, ligand; *K*, conditional stability constant; CLE-ACSV, competitive ligand exchange adsorptive cathodic stripping voltammetry; SA, salicylaldoxime.

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contrasts sharply with ammonia-oxidizing bacteria (AOB), the other major ammonia oxidizers (Ensign et al., 1993), which use iron (Fe)-dependent metalloenzymes in their electron transport system and should therefore have a lower reliance on Cu. Several studies have also shown that AOA are often ubiquitous in oceanic sites with high nitrification activity and that they can outnumber AOB by considerable margins (Beman et al., 2010; Francis et al., 2005, 2007). These findings underscore the likely significant contributions of AOA to nitrification worldwide and the marine nitrogen cycle as a whole.

More recently, Jacquot et al. (2013) revealed the existence of a tight correlation between the depths of the primary nitrite maxima (PNM) and local Cu^{2+} minima in the oligotrophic waters of the eastern tropical South Pacific (ETSP). They postulated that this feature arose because of heavy Cu^{2+} drawdown by AOA and Fe-limited diatoms engaged in ammonia oxidation and nitrate reduction, respectively. Both processes are mediated by Cu-dependent metalloenzymes and produce nitrite (Francis et al., 2005; Maldonado et al., 2006). Peers et al. (2005) reported that Cu limitation in the oceanic diatom *Thalassiosira oceanica* (equivalent to 65% of its maximal growth rate, μ_{max}) can occur when the Cu^{2+} concentration drops below ~1.25×10^{-14} M. Amin et al. (2013) demonstrated that Cu limitation in SCM1 (76% of μ_{max}) occurs when the Cu^{2+} concentration falls below ~2×10^{-13} M. Cu^{2+} concentrations in the ETSP, particularly within the PNM, often fell well below either putative limitation threshold (Jacquot et al., 2013). Although a lack of supporting data ultimately precluded Jacquot et al. (2013) from determining whether ammonia oxidation by AOA or nitrate reduction by Fe-limited diatoms was most responsible for the feature, they argued that the ubiquity of AOA in the region (Molina et al., 2010) and their absolute dependence on Cu (Walker et al., 2010) meant that Cu limitation had the potential to impose larger constraints on ammonia oxidation.

One objective of our study was to evaluate whether the measured threshold for Cu limitation reported by Amin et al. (2013) is relevant in coastal environments. We selected a study site in Hood Canal, a long (110 km), narrow (1–2 km) sub-basin of the Puget Sound estuary in Washington (Newton et al., 2011), where AOA have recently been shown to dominate ammonia oxidation (Horak et al., 2013). Spring blooms in Hood Canal are long-lived, intense and last from early spring, or even late winter, until late summer. Bloom dynamics typically switch from light limitation in the winter to nutrient limitation – generally nitrate (Mackas and Harrison, 1997) – in the summer (Devol et al., 2011). Given the basin's high biological productivity (4 g C mg^{-2} day^{-1} on average; Newton et al., 2011) and abundant AOA (Horak et al., 2013), we anticipated a strong biological demand for Cu, particularly in the upper water column. The biological uptake of Cu is often associated with diatoms and AOA, but it may also be important for other taxa that have not been studied. A survey of the Cu requirements in the existing literature suggests that AOA are the most easily Cu-limited microbes studied to date and are therefore the focus of our study (Amin et al., 2013).

In this paper, we characterize the distribution and speciation of Cu in seawater in Hood Canal on 4 occasions from July 2011 to August 2012 along with the distribution of inorganic nutrients and ammonia oxidation rates. The objective was to determine if there was a drawdown in dissolved Cu and Cu^{2+} in areas of AOA activity and to see if the Cu^{2+} concentration might fall below the limiting threshold identified in Amin et al. (2013).

2. Methods

2.1. Study site and sample collection

Sampling for this study was performed over a 2-year period during 4 cruises aboard the R/V *Clifford A. Barnes*: CB960 (July 17–22, 2011); CB974 (May 7–13, 2012); CB980 (July 16–22, 2012); and CB985 (August 24–30, 2012) near an ocean remote chemical analyzer (ORCA) buoy (Hoodsport; latitude, 47°25′18.48″N; longitude, 123°6′45.36″W) (Fig. 1; http://www.geomapapp.org; Ryan et al., 2009) operated by the School of Oceanography at the University of Washington (http://orcabase.ocean.washington.edu/). Water samples from the surface (2 m) down to just above the bottom (115 m) were collected over several days using 10 L Teflon-coated Go-Flo bottles (General Oceanics) attached to Kevlar wire. Upon retrieval the bottles were immediately pressurized with filtered compressed nitrogen gas and the water was forced through acid-cleaned Teflon tubing and acid-cleaned 0.2 μm Acropak capsules (Pall Corporation) into 1 L fluorinated low-density polyethylene (FLPE) bottles (Nalgene, Nalge Nunc International) and 250 mL low-density polyethylene (LDPE) bottles (Nalgene, Nalge Nunc International) on deck for speciation and dissolved analyses, respectively. The bottles were washed in a sequential 4-step process: 1) soaked for at least 24 h in a 5% Citranox acid detergent bath (Alconox), 2) soaked for at least 24 h in a 10% hydrochloric acid bath (HCl; Van Waters and Rogers (VWR) International), 3) filled with 10% HCl and baked at 60 °C upright and upside down (to properly leach the threads around the cap) for at least 48 h, and 4) filled with 0.1% trace metal grade HCl (Optima, Fisher) and baked at 60 °C again for at least 48 h. In between each step the insides and outsides of the bottles were thoroughly rinsed at least 5 times with Milli-Q water (18.2 MΩ; Millipore). As a final measure, the bottles were rinsed at least 5 times with sample seawater prior to collection to ensure that all the acid had been removed. The 125 mL polycarbonate bottles (Nalgene, Nalge Nunc International) used to collect samples for the ammonia oxidation rate measurements were also washed with 10% HCl and rinsed multiple times with Milli-Q water.

Following the CB974 cruise, two freshwater samples were also collected on May 14, 2012, at 13:00 PST by hand in the same acid-cleaned 1 L FLPE bottles from the North Fork Skokomish River upstream

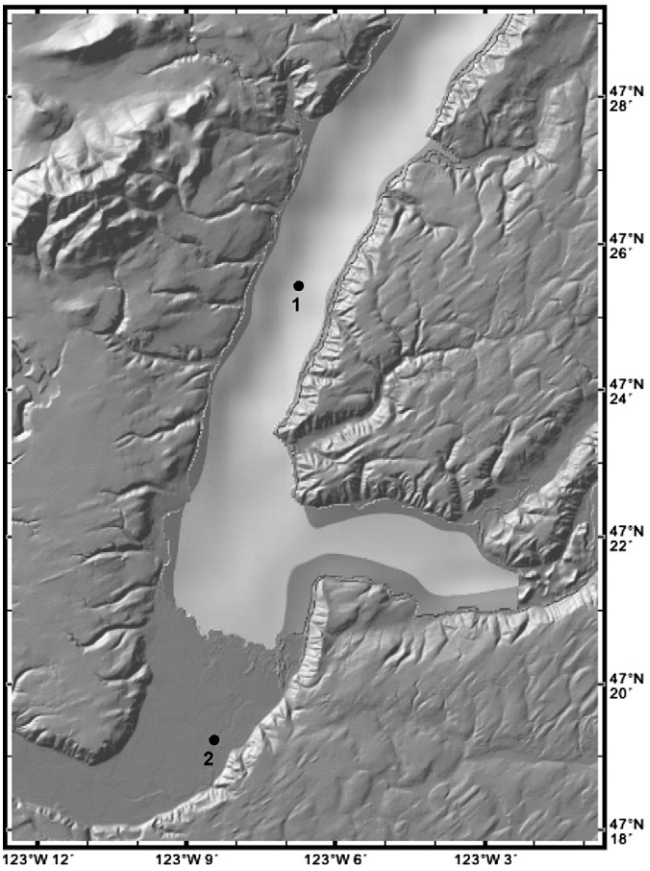

Fig. 1. Map of the study area showing the Hoodsport buoy (1) and the North Fork Skokomish River sampling sites (2). The map was created in GeoMapApp (http://www.geomapapp.org).