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Plankton copper requirements and uptake in the subarctic Northeast Pacific Ocean

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ABSTRACT

We undertook the first measurements of metabolic Cu requirements (net Cu:C assimilation ratios) and steady-state Cu uptake rates (ρCu_{ss}) of natural plankton assemblages in the northeast subarctic Pacific using the short-lived radioisotope ^{67}Cu. Size-fractionated net Cu:C assimilation ratios varied ~3 fold (1.35–4.21 μmol Cu mol C^{-1}) among the stations along Line P, from high Fe coastal waters to the Fe-limited open ocean. The variability in Cu:C was comparable to biogenic Fe:C ratios in this region. As previously observed for Fe uptake, the bacterial size class accounted for half of the total particulate ρCu_{ss}. Interestingly, carbon biomass-normalized rates of Fe uptake from the siderophore desferrioxamine B (DFB) (ρFe_{DFB}; a physiological proxy for Fe-limitation) by the >20 μm size class were positively correlated with the intracellular net Cu:C assimilation ratios in this size class, suggesting that intracellular Cu requirements for large phytoplankton respond to increased Fe-limitation. At Fe-limited Ocean Station Papa (OSP), we performed short-term Cu uptake (ρCu_L) assays to determine the relative bioavailability of Cu bound to natural and synthetic ligands. Like the volumetric ρCu_{ss} measured along Line P, the bacterial size class was responsible for at least 50% of the total ρCu_L. Uptake rates of Cu from the various organic complexes suggest that Cu uptake was controlled by the oxidation state of the metal and by the metal:ligand concentration ratio, rather than the concentration of inorganic species of Cu in solution. Collectively, these data suggest that Cu likely plays an important role in the physiology of natural plankton communities beyond the toxicological effects studied previously.

1. Introduction

Dissolved Fe concentrations in the open ocean are extremely low (Johnson et al., 1997), and Fe availability restricts phytoplankton primary productivity in major oceanic regions (for a review see Boyd et al., 2007). In response to chronic Fe deficiency, organisms inhabiting low Fe waters have evolved physiological mechanisms to reduce their Fe requirements (Brand, 1991; Sunda et al., 1991; Maldonado and Price, 1996) and increase their ability to acquire Fe. Open ocean phytoplankton have been shown to reduce their photosynthetic Fe requirements by by lowering their PSI/PSII ratio (Strzepek and Harrison, 2004), and by using electron transfer proteins with alternative trace metal catalysts (Cu-containing plastocyanin instead of the Fe-containing cytochrome c_6; Peers and Price, 2006). In addition, Fe-limited marine phytoplankton up-regulate a high-affinity Fe transport system

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(HAFeT), involving the activity of surface-bound ferric reductases (Soria-Dengg and Horstmann, 1995; Maldonado and Price, 2000, 2001), multi-Cu containing oxidases (Peers et al., 2005; Maldonado et al., 2006), and Fe permeases. This HAFeT allows phytoplankton to access organically bound Fe, the most abundant dissolved Fe species in seawater (Gledhill and van den Berg, 1994; Rue and Bruland, 1995). Collectively, these studies suggest that Cu may play a critical role in the physiology of open ocean phytoplankton, and that Cu requirements may be higher when the organisms experience Fe-limitation. Indeed, recent investigations in our laboratory have demonstrated that oceanic diatoms have significantly higher intracellular Cu quotas than coastal isolates, and that, for some species, cellular Cu demands increase 2–3 fold in response to Fe-limitation (Annett et al., 2008). Even though net Cu:C accumulation ratios of natural phytoplankton populations do not exist, calculated Cu:C ratios—calculated from the slope of the relationship between dissolved Cu and PO_4^{3-} concentrations in oceanic nutriclines, and the C normalization of this slope ($[Cu]_M/[P]_M$) using the Redfield ratio (106 mol C: 16 mol N: 1 mol P)—reveal that the cellular Cu requirements of phytoplankton in Fe-deficient waters may be significantly higher than those of other oceanic regions (7.7 vs. 4.4 μmol Cu mol C^{-1}, respectively; Annett et al., 2008). These results suggest that the Cu demand of indigenous phytoplankton may be significantly elevated in low Fe waters, possibly because of the increased physiological requirement of Cu in plastocyanin (a Cu-containing substitute for cytochrome c_6 in the photosynthetic apparatus) and the MCO of the HAFeT (Peers et al., 2005; Maldonado et al., 2006).

Given the potential role of Cu in the physiology of open ocean phytoplankton, it is imperative to understand Cu nutrition and acquisition mechanisms of natural phytoplankton populations, particularly in Fe-limited regions. As with other bioactive trace elements (e.g. Fe, Zn), 98% of Cu is complexed by organic ligands, presumably of biological origin, resulting in low inorganic metal concentrations (Moffett et al., 1990; Sunda and Hanson, 1987; Coale and Bruland, 1988, 1989; Gledhill and van den Berg, 1994; Rue and Bruland, 1995). Although pioneering lab work in metal acquisition by marine phytoplankton suggested that only inorganic metal complexes were bioavailable (e.g. Anderson and Morel, 1982; Hudson and Morel, 1990; Sunda and Huntsman, 1995), recent studies demonstrate that organic metal complexes of both Fe and Cu are also available for uptake (Hutchins et al., 1999; Maldonado and Price, 1999, 2001; Quigg et al., 2006). Rigorous physiological and genomic studies on Fe acquisition have demonstrated organic Fe utilization by a number of diatom species (Maldonado and Price, 2000, 2001; Shaked et al., 2005; Maldonado et al., 2006; Kustka et al., 2007). In contrast, the evidence for organically bound Cu utilization by marine phytoplankton remains restricted to theoretical calculations, demonstrating that the rate of inorganic Cu supply to the cell surface is slower than observed cellular uptake rates (Hudson, 1998; Croot et al., 2003; Quigg et al., 2006). Furthermore, most experimental studies on Cu acquisition have thus far been restricted to a few synthetic ligands, namely the carboxylate-containing ligands, ethylenediamine tetraacetic acid (EDTA) and NTA. These synthetic ligands likely differ significantly from natural Cu complexing ligands, which are hypothesized to contain S and N donor groups (Leal and van den Berg, 1998; Croot et al., 2000; Laglera and van den Berg, 2003; Dupont et al., 2006; Wiramanaden, 2007), with varying conditional stability constants (Coale and Bruland, 1988; Moffett, 1995; Croot et al., 1999; Dryden et al., 2004; Moffett and Dupont, 2007). So far, only one study has investigated acquisition of Cu from natural ligands by laboratory monocultures (Quigg et al., 2006).

The Northeast Pacific Ocean is a well-studied Fe-limited oceanic region, with low phytoplankton biomass and primary productivity (Boyd, 2004). For the last five decades, oceanographic observations at stations along Line P, a seaward transect from the coast of British Columbia to Ocean Station PAPA (OSP), have provided a detailed understanding of ocean processes in this region (Peña and Bograd, 2007). Line-P includes five major stations, from P4, in the high Fe waters off the coast of British Columbia, to P26, in the Fe-deficient waters of OSP (Johnson et al., 2005). This presents a unique opportunity to investigate Cu demands and acquisition by phytoplankton communities along a natural Fe gradient. The purpose of this field investigation was to determine—using the short-lived, gamma emitting radionuclide ^{67}Cu—the Cu requirements of indigenous plankton populations along Line P, and the bioavailability of organically bound Cu at OSP.

2. Materials and methods

2.1. Water collection

Work was conducted on board J.P. Tully cruise 2006-15 from Sept 21 to Sept 30, 2006. For the experiments, unfiltered seawater was collected at five stations along Line P (Table 1) using a trace metal clean Teflon peristaltic pump and tubing system suspended with Kevlar line (Johnson et al., 2005). Water was sampled from 10 m depth at all stations except P4 (25 m), with both depths well within the mixed layer. All polycarbonate bottles were successively soaked in 50% HNO_3 (Environmental grade; Fisher) and 50% HCl (VWR) for one week. In between each acid treatment, the bottles were rinsed with 18 MΩ cm^{-1} Milli-Q water (Millipore; hereafter referred to as MQ) inside a positively pressurized trace metal clean room. The bottles were then partially filled with 0.1% HNO_3 (Trace metal grade; Fisher) and triple-bagged prior to embarking on the cruise. Upon reaching each station, the polycarbonate bottles were emptied and subsequently rinsed three times with unfiltered seawater pumped directly into a laminar flow hood from the desired sampling depths. The 1 or 2 L polycarbonate bottles were then filled with unfiltered water, and stored briefly (for a maximum of 30 min) in the dark at 4 °C prior to starting all experiments.

2.2. Biological and chemical analysis

Samples for size fractionated chlorophyll *a* (chl *a*) were collected using Poretics polycarbonate filters (porosities of