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Temporal dynamics and depth variations of dissolved free amino acids and polyamines in coastal seawater determined by high-performance liquid chromatography

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ABSTRACT

Short-chained aliphatic polyamines (PAs) are a class of labile dissolved organic nitrogen (DON) that has biogeochemical similarities to dissolved free amino acids (DFAAs). Here we investigated the relative contributions of DFAAs and PAs to the total DON pool and their diurnal dynamics at different depths at the Gray's Reef National Marine Sanctuary (GRNMS) in the spring and fall of 2011. A high-performance liquid chromatography (HPLC) method that uses pre-column fluorometric derivatization with *o*-phthaldialdehyde, ethanethiol, and 9-fluorenylmethyl chloroformate was optimized to measure 20 DFAAs and 5 PAs in seawater simultaneously. The concentrations of DFAAs and PAs varied over 5-fold during individual diurnal cycles and between seasons; and concentrations of the former (tens to hundreds nM) were typically at least one order of magnitude higher than the latter (a few nM). An exception was noted in fall surface water samples when the total PAs reached 159 nM and the ratio of PAs to DFAAs was about 2:3. Compositions of individual DFAAs and PAs also exhibited temporal dynamics, with glycine and spermidine consistently the most abundant compound in each pool, respectively. DFAA concentrations appeared to track chlorophyll *a*, whereas, total PA concentrations were strongly correlated with bacterial cell abundance. Our results indicate that, at least occasionally, PAs may serve as an important DON pool at the GRNMS. This view is in accordance with recent molecular data but contrasts to measurements made in some other marine environments.

1. Introduction

Dissolved organic nitrogen (DON) represents a major pool of fixed nitrogen in marine systems and serves as an important nitrogen and carbon source for marine bacterioplankton (Fuhrman and Ferguson, 1986; Bronk et al., 1994; Berman and Bronk, 2003). Dissolved free amino acids (DFAAs) are recognized as an important component of labile marine DON that originates primarily from phytoplankton cells via active exudation, during the process of cell senescence, or upon sloppy feeding by zooplankton (Webb and Johannes, 1967; Carlucci et al., 1984; Rosenstock and Simon, 2001). Once in seawater, DFAAs are rapidly transformed by bacteria (Kirchman and Hodson, 1986), a process that can sustain over 100% of the estimated N demand of marine bacteria (Keil and Kirchman, 1991; Jørgensen et al., 1993) and contributes to the low DFAA concentrations (<1–10 nM) that are typically found in seawater (Mopper and Lindroth, 1982; Fuhrman and Ferguson, 1986). Therefore, although DFAAs only make up a small proportion of the total DON pool, they contribute significantly to the DON flux (Lee and Bada, 1975; Tada et al., 1998; Berman and Bronk, 2003).

Short-chained polyamines (PAs), such as putrescine, spermidine, and spermine, are another group of labile DON compounds that share many important biogeochemical features with DFAAs. First, PAs are also found ubiquitously in all living organisms, with phytoplankton as their major source in marine ecosystems (Lee and Jørgensen, 1995). Second, concentrations of PAs inside phytoplankton cells (μM to mM; Tabor and Tabor, 1985; Lu and Hwang, 2002) and in seawater (nM; Nishibori et al., 2001a, 2001b, 2003) are both comparable to those of DFAAs. Finally, radiotracer experiments and recent gene-based studies have consistently suggested that, like DFAAs, PAs may serve as an important source of C, N, and/or energy to marine bacterioplankton (Höfle, 1984; Lee and Jørgensen, 1995; Poretsky et al., 2010; Mou et al., 2011). However, PAs are historically understudied and have rarely been included in measurements of marine DON compounds. Consequently, the importance of PAs relative to DFAAs and to the total marine DON pool has not been rigorously established.

One factor contributing to this knowledge gap is the lack of effective analytical methods that can simultaneously quantify DFAAs and PAs in seawater, even though methods specifically targeting either marine DFAAs (Mopper and Lindroth, 1982) or PAs (Nishibori et al., 2003) are

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available. Simultaneous analyses of DFAAs and PAs, using high-performance liquid chromatography (HPLC), have been reported for samples of cheese, wine, beer, and vinegar (Kutlán and Molnar-Perl, 2003; Körös et al., 2008). However, these methods were developed for food extracts, which typically contain nearly 1000-fold higher concentrations of PAs and DFAAs (μM levels) than natural seawater (nM levels). Moreover, the effect of high salts in seawater samples on the sensitivity and accuracy of these methods is unknown.

The objective of this study is two-fold: 1) to optimize current HPLC methods for simultaneous and sensitive measurements of DFAAs and PAs in seawater, and 2) to compare the abundance of DFAAs and PAs and examine their temporal dynamics at different depths in a near-shore site on the continental shelf of the South Atlantic Bight.

2. Methods

2.1. Study site and sampling procedure

The sampling site is located off the coast of Georgia within the Gray's Reef National Marine Sanctuary (GRNMS; 31° 24.04′ N, 80° 51.51′ W). Two diurnal sampling series were conducted on-board the R/V Savannah in 2011, one in spring (April 21–22) and the other in fall (October 5–6). Water samples were collected every 3 h during a 24-hour period on each cruise (8 casts in each season) using Niskin bottles mounted on a rosette sampling system (Sea-Bird Electronics, Bellevue, WA). Depth profiles of environmental variables including temperature, salinity and photosynthetically active radiation (PAR) were measured in situ with a conductivity–temperature–depth (CTD) water column profiler (Sea-Bird Electronics, Bellevue, WA) that was also mounted on the rosette sampling system. The water column was stratified in spring, so samples were taken at nominal depths of 2 m (referred to as surface water hereafter), 4 m (within the thermocline, referred to as mid-depth hereafter) and 17 m (~2.5 m above the sediment–water interface, referred to as bottom water hereafter) (Fig. 1A). There was no thermocline present in fall and samples were taken at depths of 2 m (surface) and 17 m (bottom) (Fig. 1B).

Water samples were sequentially filtered through 3 and 0.2 μm diameter pore-size membrane filters (Pall life sciences, Ann Arbor, MI) under low vacuum pressure (~10 mm Hg) immediately after collection. The filtrates were collected in amber glass vials and stored at −80 °C before measurements of the concentrations of DFAAs, PAs, dissolved organic carbon (DOC), dissolved nitrogen (DN; including both organic and inorganic forms), nitrate/nitrite (NO_x^-), ammonium (NH_4^+) and soluble reactive phosphorus (SRP). Five hundred milliliters of water were filtered through GF/F filters (Whatman International Ltd, Maidstone, England), which were immediately wrapped in aluminum foil and stored at −20 °C for chlorophyll *a* (Chl *a*) measurements. Bacterioplankton that passed 3 μm diameter pore-size membrane filters were fixed with 1% freshly prepared paraformaldehyde and incubated at room temperature for 1 h. Afterwards, fixed cells were collected onto 0.2 μm diameter pore-size polycarbonate membrane filters and stored at 4 °C before cells were enumerated.

All samples were prepared in triplicate. Glassware, GF/F filters and aluminum foil were combusted at 500 °C for at least 6 h before use.

2.2. HPLC analysis

Simultaneous measurements of 20 individual DFAAs, 5 individual PAs, and ammonium (Table 1) were performed on a Prominence 20A HPLC system (Shimadzu Corp., Tokyo, Japan) consisting of a SIL-20A autosampler, an LC-20AD quaternary pump, a CTO-20A column oven, and an RF-20Axs fluorescence detector, using a protocol modified from a procedure developed for analysis of cheese (Körös et al., 2008). Briefly, standard solutions of DFAAs and PAs were prepared using HPLC-grade water. 10 μL of α-aminobutyric acid (AABA) and 1,7-diaminoheptane (DAH) mixture (5 μM each) were added as internal standards for the quantification of DFAAs and PAs, respectively. A two-step derivatization procedure was performed off-line using *o*-phthalaldehyde (OPA), ethanethiol (ET) and 9-fluorenylmethoxycarbonyl chloride (FMOC-Cl). First, the OPA–ET reagent was freshly prepared by mixing 500 μL of OPA stock solution (0.22 g OPA in 10 mL methanol), 2 mL of 0.8 M borate buffer (pH 11.0), 52 μL ET and 7.448 mL of methanol, and then aged in dark for 90 min at 4 °C before use. Then, 15 μL of the OPA–ET reagent

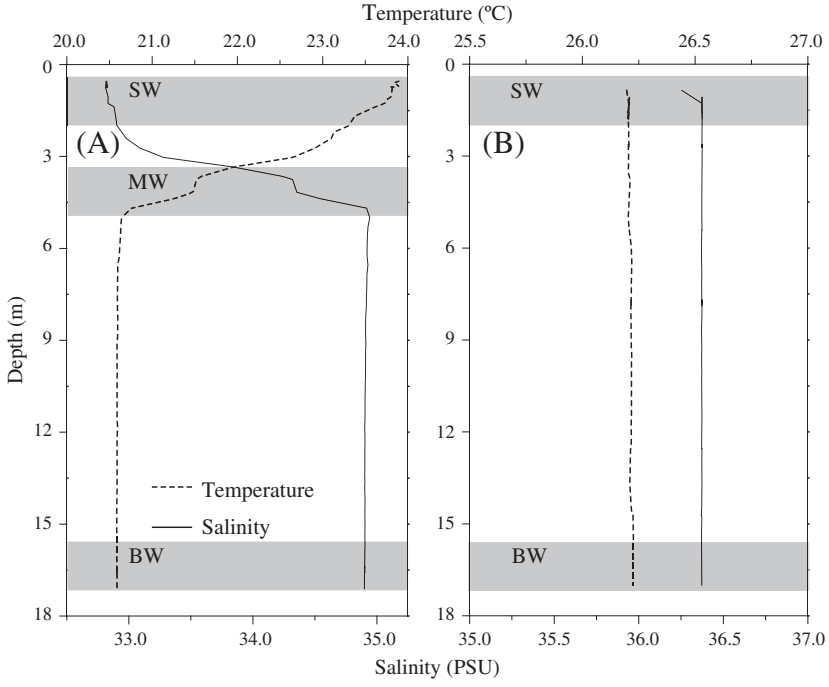

Fig. 1. Depth profiles of temperature and salinity at the GRNMS in (A) spring (April) and (B) fall (October) of 2011. Abbreviations: SW, surface water; MW, mid-depth water; and BW, bottom water.