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Ultrahigh resolution mass spectrometric differentiation of dissolved organic matter isolated by coupled reverse osmosis-electrodialysis from various major oceanic water masses

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ABSTRACT

A high-recovery technique of dissolved organic matter (DOM) isolation – reverse osmosis coupled with electrodialysis (RO/ED) – was used to isolate DOM from the North Atlantic Senegal-Mauritanian upwelling area surface water (5 m), North Atlantic oxygen minimum water (415 m) and deep water (3000 m), North Pacific subtropical gyre surface water (5 m), and North Pacific intermediate water (674 m) and deep water (3500 m). Samples were characterized by ultrahigh resolution Fourier transform ion cyclotron resonance mass spectrometry with electrospray ionization (ESI FTICR-MS). RO/ED isolated DOM samples share a significant number of common formulas accounting for 54–79% of formulas in each sample. Total dissolved carbohydrate (TCHO) concentrations in RO/ED isolated DOM were specifically measured using a colorimetric method, and were found to have higher contribution to DOC than estimated by FTICR-MS data. Percentages of TCHO-C in DOC are in the range of 3.7–19.6% in all samples, with the North Pacific deep (3500 m) water having the lowest % and the North Atlantic upwelling core surface water having the highest %. Principal component analysis (PCA) using the relative magnitudes of MS peaks facilitated identification of specific peaks that are enriched in different samples. Peaks enriched in surface samples have higher H/C values than peaks enriched in deep samples, in both the North Atlantic DOM and the North Pacific DOM. This enrichment pattern is likely due to the selective photo-degradation of aromatic compounds and the bio-production of aliphatic and carbohydrate-like compounds in surface waters, and the selective bio-degradation of aliphatic and carbohydrate-like compounds with increasing depth. In further support of a photo-degraded signature for DOM in surface waters, photo-resistant and photo-produced molecular formulas were present in the highest numbers in the surface North Pacific subtropical gyre DOM. Peaks enriched in the North Pacific intermediate and deep DOM have significantly higher O/C values than the North Atlantic oxygen minimum layer and deep DOM, for both CHO formula compounds and CHON formula compounds. This difference in O/C values observed for the deep Pacific vs. Atlantic suggests oxidation of DOM, possibly via microbial activity during the ageing of DOM or the preferential remineralization of DOM from sinking particles at depth in the Pacific.

1. Introduction

Marine dissolved organic matter (DOM) is important to numerous key oceanic biogeochemical processes because it is one of the largest pools of dynamic carbon on the earth (Hedges, 1992). The marine DOM pool is composed of a tremendous number of molecules of varying complexity derived from biota present in the water, molecules from organisms that have previously inhabited the water, and chemically and biologically altered autochthonous and allochthonous biomolecules (Carlson, 2002). The detailed composition and structure of marine DOM is a potential treasure-trove of information relating to its source, reactivity and fate, as well as its alterations during transport (Hedges, 1992).

A complete detailing of the chemical composition of DOM, and its origin, structure and function in the global carbon cycle is hampered by the limited application of advanced techniques to desalt, concentrate, isolate and then molecularly characterize marine DOM. The commonly

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used techniques to concentrate and isolate marine DOM are tangential-flow ultrafiltration (e.g. Benner et al., 1992; Buesseler et al., 1996) and solid phase extraction (SPE) with XAD resins (e.g. Lara and Thomas, 1994; Meyers-Schulte and Hedges, 1986; Stuermer and Harvey, 1977), C_{18} material (e.g. Kim et al., 2003; Sleighter and Hatcher, 2008) or PPL (e.g. Dittmar et al., 2008), which all fractionate the total marine DOM pool to varying degrees (Mopper et al., 2007). Here we have applied a recent technique using reverse osmosis coupled with electrodialysis (RO/ED), which can isolate an average of 75% of marine DOM (Green et al., 2014; Koprivnjak et al., 2009), to collect DOM samples from various major oceanic water masses. Spectroscopic evidence suggests that the DOM extracted by this technique is less fractionated (i.e, more representative) than DOM extracted by other techniques (Helms et al., 2013; Koprivnjak et al., 2009). The molar C/N ratios of RO/ED isolated DOM were more consistent with the original seawater DOM (Green et al., 2014; Koprivnjak et al., 2009).

Electrospray ionization combined with ultrahigh resolution Fourier transform ion cyclotron resonance mass spectrometry (ESI FTICR-MS) provided a molecular fingerprint for the DOM isolates (Sleighter and Hatcher, 2007; and references therein). ESI FTICR-MS has shown great promise in characterizing DOM at the molecular level (D'Andrilli et al., 2010; Flerus et al., 2012; Hertkorn et al., 2006; Kujawinski et al., 2004; Reemtsma et al., 2008; Sleighter and Hatcher, 2008; Stubbins et al., 2010). The ultrahigh mass resolution (average 400,000 at m/z 400) and accuracy (<1 ppm) of ESI FTICR-MS allows the resolution of 10–20 peaks at each nominal mass (Marshall and Rodgers, 2008) and the assignment of elemental formulas to each measured peak mass. Thus, ESI FTICR-MS is able to identify specific compounds within DOM, assign unique elemental formulas, and subsequently infer compositional differences among pools of DOM based on these elemental formulas. Recently, FTICR-MS has become even more powerful as multivariate statistical analysis approaches have been introduced and applied to explore the large data sets encountered from mass spectra of DOM (Abdulla et al., 2013; Kujawinski et al., 2009; Sleighter et al., 2010).

In this study, seven sites were selected in an attempt to capture globally relevant and biogeochemically distinct pools of marine DOM, including two distant points along the global deep ocean conveyor belt, one in the Atlantic and one in the Pacific. Two DOM samples from the surface waters (5 m, both core and edge area) of the productivity maximum of Senegal-Mauritanian upwelling in the North Atlantic were collected and were expected to represent biolabile DOM on top of background refractory DOM. Another DOM sample from the surface water (5 m) of the North Pacific subtropical gyre at the ALOHA-series site was expected to be enriched in semi-biolabile DOM and highly photo-bleached DOM on top of background refractory DOM. The remaining four DOM samples were collected from deep waters from the North Atlantic (415 m and 3000 m, respectively) and the North Pacific (674 m and 3500 m, respectively) and were expected to be enriched in background refractory DOM. This focused sampling approach differs from the recent work of Flerus et al. (2012), which utilized low volume, high throughput PPL SPE extraction to examine a larger set of water samples from the Atlantic Ocean. The focused approach we employed was necessitated by the cost and time required for processing of RO/ED-collected DOM (10–12 hours per ~200 L sample).

2. Experimental methods

2.1. Sample collection

Four sites in the eastern North Atlantic Ocean were sampled aboard the R/V *Oceanus* during Cruise OC449-3 in September 2008. Water was collected from Niskin bottles on a CTD rosette. The samples include North Atlantic deep water (3000 m), oxygen minimum layer water (415 m) and two samples of high productivity surface seawater (5 m) from the Senegal-Mauritanian upwelling zone: one on the edge and one in the core region. The water from the core area had a lower temperature but higher chlorophyll fluorescence than the edge sample (Table 1). Water from the North Pacific Ocean was collected aboard the R/V *Kilo Moana* at the ALOHA Hawai'i Ocean Time series site (http://hahana.soest.hawaii.edu/hot/) in September 2009 from near surface (5 m) in the subtropical gyre and from North Pacific Deep Water (NPDW; 3500 m). Intermediate water (674 m) from the North Pacific Ocean was collected at the Natural Energy Laboratory of Hawaii Authority (NELHA). At NELHA, seawater was pumped through a 1915 m long, 1 m diameter high-density polyethylene pipeline into the laboratory where it was filtered and processed by RO/ED. Multiple 674 m water samples were processed by RO/ED, using approximately 200 L of initial seawater per day, in order to examine sampling variability of the RO/ED technique (see Green et al., 2014 for more information on the site and the collection of these samples). Table 1 lists relevant oceanographic parameters for each sample. These samples were also characterized by nuclear magnetic resonance (NMR) spectroscopy by Helms (2012) except the North Atlantic Senegal-Mauritanian upwelling edge region sample. The North Pacific samples were named as ALOHA samples in Helms (2012). The absorbance, fluorescence and photoreactivity of a NELHA deep water sample are presented in Helms et al. (2013).

2.2. The isolation of DOM

Samples were filtered through a 0.1 μm pore-size polyethersulfone capsule filter (Whatman Polycap ™ TC). The RO/ED procedure has been described thoroughly in previous papers (Koprivnjak et al., 2009; Vetter et al., 2007). Briefly, the RO/ED system, tanks and lines were first rinsed in a once-through mode with 50 L of filtered seawater that was to be processed, after which up to 220 L of a seawater sample were transferred to the RO/ED system tank. An initial ED phase removed most (~75%) of the sea salts, subsequently a coupled RO/ED phase removed water and sea salts at the same rate (maintaining constant conductivity) until the volume of the sample had been minimized. At this point, additional seawater sample, if any, was added to the RO/ED system tank and the first two phases were repeated. A final ED phase was used to remove remaining sea salts from the concentrated sample. Conductivity of the samples decreased significantly from above 50 mS cm^{-1} to lower than 0.050 mS cm^{-1} (equivalent to 0.0004 mol L^{-1} of NaCl). Aliquots of 142–473 L of filtered seawater from each site were processed by the RO/ED system. Samples were desalted and concentrated down to about 5 L, labeled as Final sample, and stored at −20 °C prior to further analysis. After each sample, the adsorbed DOM in the RO/ED system was rinsed with a 5 L 0.01 M NaOH (Fisher Sci., certified ACS grade) solution. The rinse solutions were collected separately, labeled as Rinse sample, and frozen immediately, although they were not analyzed in the present study. The RO/ED system blank was tested by processing 200 L of artificial seawater, which was made with combusted (450 °C, 5 h) NaCl and anhydrous $MgSO_4$ (Fisher Sci., certified ACS grade) mixed with ultrapure water (Millipore, MilliQ, resistivity ~18.2 MΩcm^{-1} at 25 °C). The salts were added to ultrapure water to approach salinity 35, with a mass ratio of 64: 7 for NaCl: $MgSO_4$. Replicates (n = 3) of 200 L of artificial seawater were processed by the RO/ED system in the same manner as samples were processed. Artificial seawater DOC increased by <2.5 μMC in all of the three replicates. This increase of DOC represents <6% of the initial DOC (normally >40 μMC) in oceanic water.

2.3. DOC/TDN measurements

Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) concentrations for filtered samples and RO/ED processed final samples were determined as non-purgeable organic carbon (NPOC) by high temperature combustion (720 °C) on a Shimadzu TOC-V_{CPH} (Shimadzu Scientific Instruments). Standard calibration curves were made with