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Suspended marine particulate proteins in coastal and oligotrophic waters

Maxime C. Bridoux [a,1], Jaqui Neibauer [a], Anitra E. Ingalls [a], Brook L. Nunn [b], Richard G. Keil [a,*]

[a] School of Oceanography, Box 355351, University of Washington, Seattle, WA 98195, USA
[b] Department of Genome Sciences, University of Washington, Seattle, WA 98195, USA

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ABSTRACT

Metaproteomic analyses were performed on suspended sediments collected in one coastal environment (Washington margin, Pacific Ocean, n = 5) and two oligotrophic environments (Atlantic Ocean near BATS, n = 5, and Pacific Ocean near HOTS, n = 5). Using a database of 2.3 million marine proteins developed using the NCBI database, 443 unique peptides were detected from which 363 unique proteins were identified. Samples from the euphotic zone contained on average 2-3x more identifiable proteins than deeper waters (150-1500 m) and these proteins were predominately from photosynthetic organisms. Diatom peptides dominate the spectra of the Washington margin while peptides from cyanobacteria, such as *Synechococcus sp.* dominated the spectra of both oligotrophic sites. Despite differences in the exact proteins identified at each location, there is good agreement for protein function and cellular location. Proteins in surface waters code for a variety of cellular functions including photosynthesis (24% of detected proteins), energy production (10%), membrane production (9%) and genetic coding and reading (9%), and are split 60-40 between membrane proteins and intracellular cytoplasmic proteins. Sargasso Sea surface waters contain a suite of peptides consistent with proteins involved in circadian rhythms that promote both C and N fixation at night. At depth in the Sargasso Sea, both muscle-derived myosin protein and the muscle-hydrolyzing proteases deseasin MCP-01 and metalloprotease Mcp02 from γ-proteobacteria were observed. Deeper waters contain peptides predominately sourced from γ-proteobacteria (37% of detected proteins) and α-proteobacteria (26%), although peptides from membrane and photosynthetic proteins attributable to phytoplankton were still observed (13%). Relative to surface values, detection frequencies for bacterial membrane proteins and extracellular enzymes rose from 9 to 16 and 2 to 4% respectively below the thermocline and the overall balance between membrane proteins and intracellular proteins grows to an approximate 75-25 split. Unlike the phytoplankton membrane proteins, which are detrital in nature, the bacterial protein suite at depth is consistent with living biomass.

1. Introduction

Marine particulate organic matter (POM) is a mixture of living biomass (e.g., microalgal cells, bacteria, archaea etc.) and nonliving detritus (e.g., fecal pellets, diatom frustules, clay particles with organic coating) with a broad range of sizes, forms and diagenetic reactivities (Dong et al., 2010; Kaiser and Benner, 2008; Lee et al., 2009b). This pool of organic matter (OM) plays a major role in many oceanic and global biogeochemical processes such as the regulation of air-sea carbon fluxes, vertical transport of nutrients to deep water, fueling of marine food webs in the ocean interior and benthos, as well as transformation of particulate OM to the dissolved organic matter pool (Hedges et al., 2001; Lee et al., 2009a). Detailed characterization of its sources, forms and fates is thus essential in order to access the biogeochemical information the POM pool carries.

Hydrolyzable amino acids are the largest identified molecular fraction in marine OM (Wakeham et al., 1997), and much of this is thought to be in the form of recognizable proteins as opposed to detrital peptides or humic-type organics (Long and Azam, 1996; Saijo and Tanoue, 2005; Tanoue et al., 1995). Although analysis of this pool has historically been via hydrolysis and amino acid quantification (Kaiser and Benner, 2012; Keil and Kirchman, 1993; Nguyen and Harvey, 1997), the identification of proteins and peptides in POM promises to provide additional information about their origin and cellular function, including structural intent, energy production and metabolism. Because of this, additional tools for understanding amino acids in POM have long been sought (Tanoue, 1992), and a variety of electrophoretic and proteomic approaches have been applied (Moore et al., 2012b; Morris et al., 2010; Nunn et al., 2003, 2010; Suzuki et al., 1997; Tsukasaki and Tanoue, 2010; Yamada and Tanoue, 2003). Although applications to marine POM are still limited, the most common approach is the use of 'shotgun' metaproteomics (Moore et al., 2012b; Nunn et al., 2013), a discovery-based proteomics tool that facilitates a non-biased approach to protein identification from complete unknowns. Using the rapidly growing genomic and proteomic databases in conjunction with shotgun

* Corresponding author.
E-mail address: rickkeil@uw.edu (R.G. Keil).
[1] Currently at: Commissariat à l'énergie atomique et aux énergies alternatives, CEA, DAM, DIF, F-91297 Arpajon, France.

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proteomics can provide information on the origin and cellular localization of proteins which can shed light on mechanisms protecting proteins from degradation (Moore et al., 2012b; Nunn et al., 2010).

Environmental proteomics is a young and rapidly changing field. Metaproteomic approaches are currently not quantitative (Morris and Nunn, 2013), yet some information has been gleaned about how the method compares to traditional amino acid analyses. Positive correlations between total peptide identifications, protein concentration (Bradford assay) and total combined amino acid concentrations (Nunn et al., 2010) suggest that the factors controlling concentrations and detection are similar. Molar compositions of the peptides detected using metaproteomic are similar to that of the total hydrolysable amino acid pool (Moore et al., 2012a,b), indicating that the two approaches are likely pulling from the same pool of material. None-the-less, a quantitative comparison between metaproteomic pool and total hydrolysable amino acids is not yet possible. It is likely that there remain a vast number of proteins not-yet identified in marine samples, as advances in chromatography and mass spectrometry routinely lead to increases in the number of proteins detected in environmental samples (e.g. Yoshida et al., 2014). A method that compliments the metaproteomic approach is to use targeted proteomics to quantify peptides of interest (Bertrand et al., 2013; Saito et al., 2014). This approach, combined with further enhancements in metaprotemics, promises to dramatically change our understanding of protein cycling in the natural environment.

The use of proteomic techniques has allowed the discovery of new features of marine phytoplankton (Dyhrman et al., 2012; Jones et al., 2011; Nunn et al., 2013) and heterotrophic bacteria (Mattes et al., 2013; Morris et al., 2010; Smith et al., 2013; Sowell et al., 2009, 2011). For example, Morris et al. (2010) showed that rhodopsins and TonB-dependent transporter proteins, which are used by bacteria to generate proton gradients and assimilate nutrients, are ubiquitous in Atlantic Ocean surface waters. Moore et al. (2012b) showed that organelle-bound, transmembrane and photosynthetic proteins from diatoms are transported to depth in the Bering Sea whilst other phytoplanktonic proteins are lost from the detection window. This work echoes that of Nunn et al. (2010) who evaluated the degradation of *Thalassiosira pseudonana* under controlled lab conditions and concluded that organelle and membrane protection represent important mechanisms to enhance protein transfer to the deep sea and preservation.

We collected euphotic zone and intermediate water samples of POM from two oligotrophic environments; the Sargasso Sea and the oligotrophic north Pacific. We also sampled one coastal environment, the Washington margin in the north-east Pacific Ocean (Fig. 1). In order to evaluate potential similarities and differences between oceans and oceanic zones (e.g. depth), we performed metaproteomic analyses to characterize the suspended material. The primary purpose of the study was to confidently identify well-represented proteins in the POM fraction from these areas, determine their catalytic functionality and ascertain taxonomic information about the proteins' point of origin. Like Morris et al. (2010) transporter proteins were observed at depth in both coastal and oligotrophic waters, but rhodopsins were only detected in surface waters. This suggests that while transporter proteins are utilized by bacteria at all depths, the membrane-bound light harvesting components of phototrophic bacteria are only expressed at the surface. Like Moore et al. (2012a,b), we found that only specific types of proteins from diatoms are identified after being transported to depth, and extend this finding to include similar structures from the photosynthetic cyanobacteria of the oligotrophic ocean, suggesting that selective transport of photosynthetic proteins to depth is not limited to those derived from diatoms.

2. Material and Methods

2.1. Sample Collection

POM samples were collected *in situ* using large volume water transfer systems (McLane pumps) at five depths each for the Sargasso Sea (March 2011; R/V Atlantic Explorer), the tropical north Pacific (July 2010; R/V Kilo Moana) and the Washington margin locations (September 2010; R/V Wecoma)(Table 1, Fig. 1). During filtration, samples were passed through a large (4 mm) mesh screen onto combusted 142 mm diameter GF/F filters that were double stacked. Three replicate pumps were deployed at the same depth to compare three different filter choices, a 0.2 μm Durapore filter (Millipore Inc, polyvinylidene fluoride low-protein binding membrane), a single GF/A filter, and a double stack of GF/F filters.. After collection, filters were folded, packed in combusted aluminum foil sleeves and kept dark and frozen (-80 °C) until analysis in the laboratory, which occurred within 9 months of

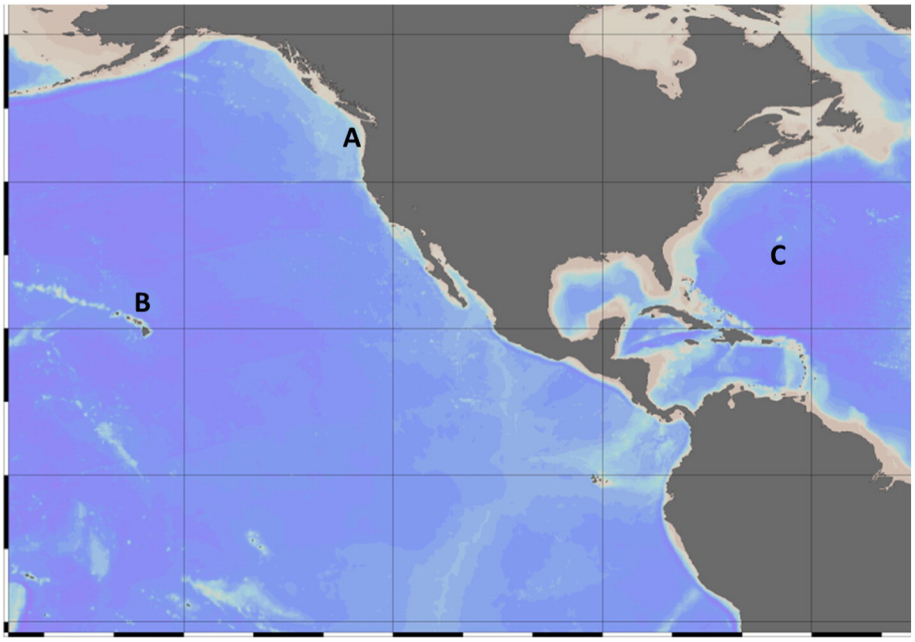

Fig. 1. Location of sampling sites for A) Northeastern Pacific (WA coast), B) North Pacific Subtropical gyre and C) the Sargasso Sea.