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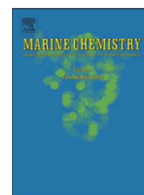

Contrasting patterns of peptidase activities in seawater and sediments: An example from Arctic fjords of Svalbard

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ABSTRACT

The structural specificities and activities of microbial extracellular enzymes help determine the nature and quantity of substrates available for microbial uptake, and therefore the rate and location of organic matter transformation in the ocean. Previous work has demonstrated major differences in the spectrum of enzymes hydrolyzing polysaccharides in the water column and sediments, particularly in high-latitude locations. To determine whether pelagic–benthic contrasts in enzyme activities extend to other classes of organic matter, the hydrolysis of five peptide substrates was investigated in two Arctic fjords of Svalbard. Substrates were intended to measure leucine aminopeptidase activity (exo-acting; terminal-unit cleaving), plus chymotrypsin and trypsin activities (endo-acting; mid-chain cleaving). All substrates were readily hydrolyzed in surface sediments. In contrast, only two to four of the peptide substrates were hydrolyzed in the water column of both fjords. Chymotrypsin activities were undetectable or were far lower than trypsin and leucine aminopeptidase activities in the water column, but were comparable to the activities of other enzymes in sediments. The pelagic–benthic contrast in the spectrum of peptide substrates hydrolyzed in these fjords parallels patterns of hydrolysis previously observed for polysaccharides. Some organic matter may pass untouched through the water column due to a lack of the specific enzymes required for hydrolysis; these substrates could then selectively fuel benthic metabolism. Pelagic–benthic contrasts in enzymatic capabilities may be widespread in the ocean, and thus affect the processing of a significant fraction of marine organic matter.

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1. Introduction

Microbial degradation of organic matter is an essential step in the marine carbon cycle. Microbial communities process an estimated half of marine primary productivity (Azam and Malfatti, 2007), transforming and respiring organic carbon, regenerating nutrients, and modifying sedimentary organic matter as it undergoes long-term burial (Arndt et al., 2013). Degradation of high molecular weight substrates is initiated by the activities of extracellular enzymes, which hydrolyze substrates to sizes sufficiently small for uptake. These enzymes thus determine the fraction and nature of substrates available to heterotrophic microbial communities. Standard methods to measure microbial enzyme activities rely on a few small substrate proxies (usually MUF-β-glucose and leucine-MCA) to obtain data that are often extrapolated to polysaccharide and protein hydrolyzing enzymes in general (e.g. Fukuda et al., 2000; Zaccone et al., 2012). These small substrate proxies are useful for intercomparisons among sites and studies, but they do not measure the activities of endo-acting enzymes with which heterotrophic microbes cleave substrates mid-chain, an essential step in hydrolysis of macromolecules (Weiner et al., 2008).

To gain insight into the structural specificities and activities of macromolecule-cleaving enzymes, we have fluorescently labeled polysaccharides as well as plankton extracts and have measured their hydrolysis in diverse regions of the ocean. A decade's worth of data revealed a latitudinal gradient in the capabilities of microbial communities in the surface ocean to hydrolyze polysaccharides of differing structure (Arnosti et al., 2011). The decreasing spectrum of enzyme activities measurable at high latitudes compared to temperate latitudes parallels reports of latitudinal gradients in microbial community composition (Baldwin et al., 2005; Pommier et al., 2007; Fuhrman et al., 2008; Sul et al., 2013), and thus suggests a direct connection between microbial community composition and function. Our previous investigations have also demonstrated striking contrasts in the spectrum of substrates hydrolyzed in the water column and sediments, with fewer substrates hydrolyzed in the water column compared to the underlying sediments (Arnosti, 2000, 2008). These contrasts in pelagic and benthic functions also parallel differences in microbial community composition that we have demonstrated in high latitude locations (Teske et al., 2011; Cardman et al., 2014).

These investigations, however, focused on hydrolysis of polysaccharides, only one of the major classes of macromolecules constituting marine organic matter. Observations of structurally-related differences in rates of peptide hydrolysis in the water column (e.g. Pantoja et al., 1997; Obayashi and Suzuki, 2005; Liu et al., 2010, 2013) raise the

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possibility that pelagic and benthic microbial communities might also exhibit different patterns of peptide hydrolysis. To investigate this possibility, a suite of substrates was used to measure exo- (leucine amino peptidase) and endo-acting (trypsin, chymotrypsin) peptidase activities in surface water, bottom water, and surface sediments of two Arctic fjords of Svalbard. These substrates had previously been used in several investigations of water column activities in temperate latitudes (e.g. Obayashi and Suzuki, 2005, 2008; Bong et al., 2013), and once in the Arctic (Steen and Arnosti, 2013), but their hydrolysis has not been compared simultaneously in sediments and in the overlying water column. The goal of this study was to determine whether the narrower spectrum of enzyme activities measurable in pelagic relative to benthic communities in Svalbard applies to peptides as well as to polysaccharides, and thus includes macromolecules constituting the majority of marine organic matter.

2. Methods

2.1. Study sites and sample collection

Seawater and sediment samples were collected in Smeerenburgfjord and Kongsfjord (Sts. J and F; Arnosti and Jørgensen, 2006) in late August 2011. A CTD cast (Seacat SBE 19 plus V2) was conducted to profile water column physical characteristics immediately prior to the collection of seawater samples via Niskin bottle. Salinity (via refractometer) and temperature (measured with electronic thermometer) were then measured in water from the Niskin bottle immediately after the bottle was brought on deck. At Stn J. (79° 42.8, 11° 05.19), the surface water (collected at 1 m) temperature was 4.1 °C, salinity 28 PSU, bottom water (collected at 211 m, total depth 216 m) temperature was 3.8 °C, salinity 33 PSU. At Stn. F (78° 54.58, 12° 16.07), surface water (1 m) was 2.7 °C, salinity was 25 PSU; bottom water (collected at 95 m, total depth was 103 m) temperature was 2.4 °C, salinity was 31 PSU. At both fjords water was stored in clean Nalgene 10 L carboys or 2 L cubitainers that were rinsed 3× with sample water prior to final sample collection.

Surficial sediments were collected via Haps corer at both stations. At Stn. J, the surface sediments (ca. 0–1.5 cm) were light brown and oxidized, and had ample worm tubes. Sediments were collected into foil bags that were loosely closed with a generous (at least 50% of total volume) headspace to ensure oxygen exposure. The sediments at Stn. F were rust-red, with distinct black vertical streaks, and a few worm tubes. Samples were stored outside on deck (ca 4 °C) during transport to the shore lab in Ny Ålesund; experiments described below were initiated within ca. 48–72 h of sample collection.

2.2. Substrates

Five 7-amido-4-methyl coumarin (MCA) labeled substrates obtained from Sigma were used in seawater and sediment incubations to assess peptide degradation. Substrates included L-leucine-7-amido-4 MCA (L-MCA) to measure the activities of leucine amino peptidase (an exo-acting enzyme that cleaves terminal amino acids from peptides/proteins), and two substrates each to measure chymotrypsin activities and trypsin activities, in order to assess activities of enzymes that cleave peptides/proteins mid-chain (endo-acting enzymes). It should be noted that in theory larger peptides could also be hydrolyzed in a stepwise manner, with an exo-acting peptidase removing terminal amino acid groups, until a final cleavage step frees the MCA fluorophore. In the following discussion, however, the assumption is made that endo-acting peptidases cleaved the trypsin and chymotrypsin substrates. The substrates ala-ala-phe-MCA and N-succinyl-ala-ala-pro-phe-MCA were used to measure chymotrypsin activities; in the following text they are referred to as AAF-chym and AAPF-chym, respectively. Boc-gln-ala-arg-MCA and N-t-boc-phe-ser-arg-MCA were used to measure trypsin activities; in the following sections they are referred to as QAR-tryp and FSR-tryp, respectively. These substrates were dissolved in DMSO, and then diluted 1:1 with milli-Q water before addition to seawater and sediments.

2.3. Experimental setup and sampling

All experimental work was carried out in a cold room at a temperature of 3.1 °C.

2.3.1. Seawater incubations

Each of the five substrates (final concentration in seawater: 100 μM) was incubated in triplicate; one incubation was carried out with autoclaved seawater as a killed control. Substrate addition levels were selected after initial experiments with L-MCA suggested that a 100 μM concentration was saturating. Fluorescence of solutions of 1 ml seawater mixed with 1 ml of 20 mM sodium tetraborate buffer (pH 9.75) was measured at excitation and emission maxima of 365 nm and 410–450 nm, respectively, using a Promega Quantifluor solid-state single-cuvette fluorimeter. Instrument drift was monitored using a solid standard; drift never amounted to more than 0.05% of total signal. In addition to the killed controls, a seawater blank (no substrate addition) was also incubated and processed in the same manner as all of the other incubations. Seawater blanks showed no detectable fluorescence; fluorescence readings from killed controls were subtracted from readings for live incubations prior to calculation of hydrolysis rates. Hydrolysis rates were calculated from fluorescence readings using a series of MCA standards in seawater. Time points were collected at 0 h, 4 h, 12 h, and 24 h for Stn. J surface and bottom water, and 0 h, 4 h, 18 h, and 24 h for Stn. F surface and bottom water incubations.

2.3.2. Slurry preparation and incubations

A slurry (1:2 sediment: seawater) was prepared for each station using surficial sediments (ca. 0–1.5 cm, the oxidized layer) and autoclaved bottom water. Controls consisted of autoclaved slurry. 5 ml portions of the slurry were dispensed into scintillation vials, with 3 live replicates and 1 autoclaved control per substrate. After substrate addition (100 μM final concentration), the slurries were mixed and incubated. At each time point (starting with time zero immediately after substrate addition), 1 ml of slurry was dispensed into an epi-tube containing 1 ml artificial seawater. The epi tubes were mixed and centrifuged. 1 ml of supernatant was pipetted from each epi tube into a cuvette containing 1 ml borate buffer. The 2 ml volume was filtered with a 0.2 μm pore-size 25 mm diameter surfactant-free cellulose acetate syringe filter to remove suspended particles, and fluorescence was measured as above. Blanks consisted of autoclaved slurry without added substrate. Sorption controls consisted of triplicate slurries to which the MCA fluorophore was added. Live incubations, killed controls, sorption controls, and blanks were incubated and measured concurrently. Hydrolysis rates were corrected for sorption of MCA flurophore over time. Timepoints were collected at zero hours (immediately after substrate addition), at 75–90 min, and at ca. 150 min. Since hydrolysis rates in sediments were very rapid, the detector was oversaturated by the fluorescence signal of some of the substrates at 150 min, so only data from the 75–90 min time point is reported below. Precise times were used for calculation of hydrolysis rates.

3. Results and discussion

3.1. Peptide hydrolysis in pelagic environments

Hydrolysis of proteins and peptides provides access to organic nitrogen as well as organic carbon for heterotrophic microbes. The importance of this process is underlined by preferential removal of amino acids relative to other organic substrates during particle sinking and early diagenesis (Wakeham et al., 1997; Lee et al., 2004), and by the

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