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Detection of hydroxamate siderophores in coastal and Sub-Antarctic waters off the South Eastern Coast of New Zealand

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ABSTRACT

In this study, we report siderophore-type compounds found in coastal and Sub-Antarctic waters within a 60 km transect off the south east coast of Otago Peninsula in New Zealand. The presence of siderophore activity was detected using chrome azurol S assay (CAS) from organic compounds extracted from ~200 to 480 L seawater by pre-concentration on XAD-16 resin. Csaky and Rioux assays indicated the presence of more than one functional type of siderophore or siderophore-like compound. Strong Fe-binding ligand (L_1) concentrations, the sum of ligands (ΣL), and their stability constants were measured from ambient seawater samples using competing ligand equilibration-cathodic stripping voltammetry (CLE-CSV). In the study area L_1 ranged from 0.23 to 0.69 nM ($\log K'_{FeL1,Fe3+} = 22.0–22.8$), whereas ΣL values ranged from 0.53 to 1.23 nM ($\log K'_{Fe\Sigma L,Fe3+} = 21.4–22.2$). Dissolved Fe (DFe) concentrations were measured by CSV and ranged from 0.22 to 0.45 nM. This is the first application of a new liquid chromatography–tandem mass spectrometry method using the natural iron-isotope pattern to characterize siderophore-type compounds isolated from natural seawater. Altogether six siderophore-type compounds with hydroxamate functionality were detected in one coastal, as well as Sub-Antartic surface and subsurface samples. This new method and the derived results provide a foundation for future investigations of sources and structures of strong ligands in the Southern Ocean and elsewhere.

1. Introduction

Iron (Fe) is the fourth most abundant element in the terrestrial environment (Drechsel and Winkelmann, 1997), however its concentration in the open ocean is extremely low (Johnson et al., 1997; Rue and Bruland, 1997; Boye et al., 2001) because of iron's low solubility at neutral pH under aerobic conditions (Kraemer, 2004). The concentration of Fe in the surface ocean ranges from 0.02 to ≤1.0 nM (Rue and Bruland, 1997; Vraspir and Butler, 2009). Interestingly, approximately 99% of Fe is bound to organic ligands (Boye et al., 2001; Vraspir and Butler, 2009). The poor bioavailability of iron is known to limit biological productivity in the vast surface ocean regions (Behrenfeld et al., 1996). This was confirmed in Fe enrichment experiments in the high nutrient low chlorophyll (HNLC) regions of the Southern Ocean (Boyd et al., 2000) and shipboard incubation experiments (Timmermans et al., 1998). In the Southern Ocean, dissolved Fe concentration ranges from 0.10 to 0.60 nM (Boye et al., 2001). In response to Fe deficiency, bacterioplankton can secrete organic compounds, known as siderophores, to solubilize and facilitate acquisition of Fe^{3+} in the environment (Vala et al., 2006). Iron–siderophore complexes increase iron bioavailability and uptake for many organisms. In bacteria, Fe–siderophore complexes are recognized by a cognate receptor on the bacterial outer membrane that transports them through the cytoplasmic membrane (Neilands, 1995; Braun and Hantke, 1997; Winkelmann, 2002). However, the iron acquisition mechanism of phytoplankton involving siderophores is not yet entirely understood (Maldonado et al., 2005) and up until now there has been no evidence that phytoplankton is actively producing siderophores (Hopkinson and Morel, 2009; Boyd and Ellwood, 2010).

Chemically, siderophores are low molecular weight (<1 kDa) organic compounds (Reid and Butler, 1991) with high Fe^{3+} affinity constants (Matzanke et al., 1989). The typical Fe^{3+} coordinating groups in siderophores are hydroxamates (e.g. ferrichrome), catecholates (e.g. enterobactin) and the α-hydroxy carboxylates (e.g. rhizoferrin) (Ito and Butler, 2005; Zawadzka et al., 2006). To form a complex with Fe, the hydroxamate functional group loses a proton from the hydroxylamine (–NOH) group and forms a bidentate bond with the carbonyl and –NOH groups (Crumbliss, 1990; Feistner et al., 1993). The catecholate functional groups loose two protons and form a five-membered ring

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with Fe through the phenolic oxygen groups at neutral to alkaline pH (Kraemer, 2004).

The conditional binding strength of laboratory-isolated siderophores ($\log K'_{FeL1,Fe3+} = 21$ to >24) are similar to reported strong iron-binding ligands (L_1-type) in seawater determined by electrochemical methods (Rue and Bruland, 1997; Witter et al., 2000; Butler and Martin, 2005; Croot and Johansson, 2000; Hudson et al., 1992). A large proportion of these ligands have also been shown to be similar in size (300–1000 Da) and to contain iron-binding functional groups similar to siderophores (Macrellis et al., 2001). It has thus been suggested that a considerable part of strong Fe-binding ligands in the ocean are in fact siderophores (Macrellis et al., 2001). In the Southern Ocean, Fe-binding ligands were found to be in excess of the dissolved Fe concentration (0.70 to 3.0 nM) (Boye et al., 2001; Croot et al., 2004; Boye et al., 2005; Buck et al., 2010). The ligand concentration is thus low enough to make their characterization challenging, especially if one considers that there is very likely to be more than one ligand present. Recent methodological advancements have enhanced our knowledge of siderophores in open ocean seawater by improving field sampling techniques, and the detection and identification of in situ iron-binding ligands (Macrellis et al., 2001; Mawji et al., 2008a, 2008b). The presence of known hydroxamate-type marine siderophores (i.e. ferrioxamine E and G) was recently confirmed via mass spectrometry-based characterization in natural seawater (Mawji et al., 2008a). Evidence suggests that more than one functional type of siderophore may be present (Macrellis et al., 2001) in iron-limited regions of the ocean like the Southern Ocean.

In this study, we identified and characterized siderophores by chemical assays, electrochemical measurements and high performance liquid chromatography coupled with tandem mass spectrometry (HPLC–MS/MS). Here, we report for the first time the presence of siderophore-type compounds in Southern Ocean waters.

2. Materials and methods

A flowchart of our method to isolate and identify siderophores by tandem mass spectrometry, supported by chemical assays and electrochemical analysis of dissolved iron and iron-binding ligands is shown in Fig. 2.

2.1. Sampling

Sample collection was completed along the Otago Shelf transect, which has been monitored for carbon dioxide flux by Currie et al. (2009) for over a decade, and is thus very well characterized. The transect runs about 60 km off the South Island's east coast in New Zealand and cuts across four distinct water masses (Fig. 1). Moving from the coast to offshore water, the transect crosses first neritic water (0–15 km from the peninsula), then modified subtropical waters of the Southland Current (15–45 km), with the subtropical front (STF) as its eastern boundary, before entering the Sub-Antarctic surface water (SASW) at approximately 45 km (Currie et al., 2009; Sutton, 2003). The physical properties of the water masses navigated by the transect stations are generally characterized by a strong temperature gradient from STF to SASW (Uddstrom and Oien, 1999; Currie et al., 2009). The biochemical characteristics of water along the transect can be described by increasing nutrients and decreasing chlorophyll biomass from coastal to Sub-Antarctic waters (Hawke, 1989; Croot and Hunter, 1998; Murphy et al., 2001; Boyd et al., 2004).

Surface seawater samples (~200 to 480 L) were collected on board the *R/V Polaris II* in coastal water in May 2007 (sample ID NM) and July 2007 (sample ID NJ) and Sub-Antarctic waters in July 2007 (sample ID SA). Coastal water sampling for this study integrated neritic water and water of the Southland Current. Collection for the Sub-Antarctic water commenced approximately 40 km offshore. The change in temperature and salinity along the transect was monitored to distinguish the coastal from SA water. A general example of this T/S relationship with distance from shore is shown in Currie et al. (2009) (Fig. 2 therein). Exact sampling positions and oceanographic data of the samples are given in Tables 1 and 3 and Fig. 1.

Surface samples were collected using an acid-cleaned Teflon pump (Almatec) with polyethylene (PE) tubing attached to a powder-coated metal torpedo-like frame (referred to as a 'fish') that holds the tubing at the desired depth below surface water. The fish was deployed at 2–3 m depth while the boat was underway at approximately 5 knots. The Teflon pump was connected to a compressor and the pressure was set between 4 and 6 bar. The flow rate of the water was maintained between 1.0 and 1.5 L/min. Large volume samples for siderophore isolation were taken while steaming along the transect and samples for dissolved iron (DFe)

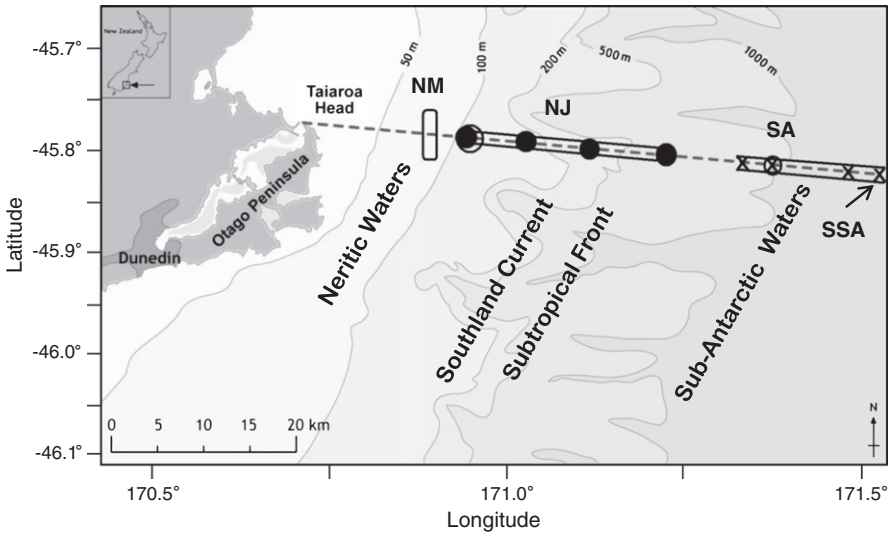

Fig. 1. Map showing the Otago Shelf transect and all sampling sites. NM sampling area for all samples (▢). Large volume in-line pre-concentration of organic ligands were taken on the inward and outward leg along the transect (– – – – –) for samples NJ and SA for. Discrete DFe sampling sites for NJ (●), and for SA(X). Discrete sampling sites for ligand concentration and stability constant analysis by electrochemistry are marked with ○. The eastern most site of sample SA is also site for subsurface sample (SAS). Precise longitudinal positions are given in Table 1, while all latitudinal positions were along the transect line.