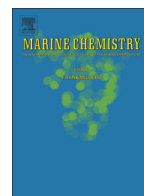




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## Iron associated with exopolymeric substances is highly bioavailable to oceanic phytoplankton

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### ABSTRACT

Growth limitation of marine algae due to lack of iron occurs in up to 40% of the global ocean. Despite important advances on the impact of organic compounds on iron biogeochemistry, their roles in controlling iron availability to prokaryotic and eukaryotic phytoplankton remain unclear. Whether algal and bacterial exopolymeric substances (EPS) include organic ligands which may help iron-limited phytoplankton growth remains an unknown. If so, then EPS could relieve phytoplankton iron limitation with implications for the biological carbon pump and hence the regulation of atmospheric CO<sub>2</sub>. Here we compared the biological impact of algal, bacterial and in situ EPS with model compounds, a siderophore and two saccharides on biological parameters including, iron bioavailability, phytoplankton growth, photo-physiology and community structure. Laboratory and field experiments demonstrated that EPS produced by marine microorganisms are efficient in sustaining biological iron uptake as well as algal growth, and can affect natural phytoplankton community structure. Our data suggest that natural phytoplankton growth enhancement in the presence of EPS was not solely due to highly bioavailable iron forms, but also because EPS contains other micronutrients. Stronger ligands were detected following iron-siderophore enrichments (log K<sub>Fe-L</sub> = 12.0) and weaker ligands were measured in the presence of EPS (log K<sub>Fe-L</sub> = 10.4–11.0). The trend of the conditional stability constants of organic ligands did not seem to be affected as a result of biological activity and photo-chemistry during our four day incubations. The shift in the phytoplankton community observed during our field experiments was not uniformly observed between different sites rendering it difficult to extrapolate which functional group(s) would benefit the most from iron bound to EPS.

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### 1. Introduction

The parameters that control iron bioavailability to phytoplankton are not well understood, but are strongly influenced by the physical and chemical forms of iron, its biogeochemical cycling, and the various iron requirements and uptake strategies of bacterio- and phytoplankton communities (e.g., Barbeau et al., 2001; Hutchins et al., 1999; Maldonado et al., 2005; Strzepek et al., 2005; Sunda and Huntsman, 1995). In addition a complex feedback exists between microorganisms and iron, where organic ligands can be biologically produced and impact iron chemistry with potential consequences for its bioavailability (e.g., Hassler et al., 2011a).

As dissolved iron (dFe) is mainly bound to organic ligands (>99%) in the ocean, it is widely accepted that these ligands control iron biogeochemistry and bioavailability. Past studies have highlighted a link

between phytoplankton iron limitation, biological iron uptake rates, iron requirement for growth and the presence of in situ iron-binding ligands (Gledhill and Buck, 2012; Hassler et al., 2012a; Morrissey and Bowler, 2012; Shaked and Lis, 2012 for recent reviews). Bacterially produced siderophores, which are excreted under iron-limited conditions, are commonly associated with strong iron-binding organic ligands translating to high conditional stability constants (Gledhill and Buck, 2012). Because siderophores are specifically recognized by bacteria, they allow bacterial regulation of iron-limitation (Granger and Price, 1999; Morrissey and Bowler, 2012). However, it has been demonstrated that some eukaryotic phytoplankton use iron bound to siderophores via reductive dissociation (Maldonado and Price, 2000), making the role of siderophores more complex than a simple bacterial switch. To date no eukaryotic phytoplankton have been found to excrete siderophores, thus raising the question about their ability to widely benefit from such bacterially-derived organic compounds for growth. Other organic ligands such as porphyrins, humic acids and saccharides could also control the growth of planktonic micro-organisms (Gledhill and Buck,

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2012; Hassler et al., 2012a; Shaked and Lis, 2012). In the ocean, a mixture of organic ligands is likely to be present. Indeed the widely unresolved nature of organic ligands in the ocean limits our ability to identify key components for the control of iron limitation in eukaryotic phytoplankton (Gledhill and Buck, 2012; Hassler et al., 2012a; Shaked and Lis, 2012).

In the open ocean, far from any continental margin, biological activity is likely a significant source of organic ligands. Most micro-organisms excrete material – often regrouped under the term exopolymeric substances (EPS) (Decho, 1990; Hoagland et al., 1993; Aluwihare et al., 1997). EPS are poorly defined macromolecules bearing various functional groups, which encompass part of the neutral and anionic polysaccharides, amino acids, and protein reported in the ocean (e.g. Biersmith and Benner, 1998; Mancuso Nichols et al., 2005). As a result of surface reaction and aggregation, EPS are also found in marine transparent EPS and marine snow (Santschi et al., 2003). As such, EPS can represent up to 50% of marine dissolved and colloidal organic carbon (Aluwihare et al., 1997; Verdugo et al., 2004).

Saccharides and bacterial EPS have been demonstrated to bind iron and enhance its solubility as well as its biological uptake (viz. bioavailability) to Southern Ocean phytoplankton (Rue and Bruland, 2001; Hassler and Schoemann, 2009a, 2009b; Benner, 2011; Hassler et al., 2011a, 2011b). In addition, eukaryotic phytoplankton such as diatoms and the haptophyte *Emiliania huxleyi* have been shown to produce iron-binding organic ligands with a conditional stability constant ( $K_{Fe'L}$ ) of  $10^{11.5}$  (Boye and van den berg, 2000; Rue and Bruland, 2001; Rijkenberg et al., 2008). Finally, organic ligands have been shown to be produced in the iron-depleted Southern Ocean in response to in situ iron enrichment experiments (Rue and Bruland, 1997; Boye et al., 2005). This paper is a companion study to the composition and the impact of marine EPS on iron chemistry (Norman et al., in press). The study from Norman et al. demonstrated that material excreted by bacterial and phytoplankton cultures, as well as by natural blooms, contribute to the pool of marine iron-binding ligands with stability constants ( $K_{Fe'L} = 10^{11.2}–10^{11.9}$ ) that were similar to what has been reported for the ocean since 20 years (reference herein). Moreover, EPS contributed to the pool of humic-like material as per Laglera and van den Berg (2009) and increased iron solubility. Finally, EPS did not contain only iron but also other nutrients (N, P, Co, Cd, Zn) that are reported to limit phytoplankton growth in some parts of the ocean. However, the impact that EPS from different origins exert on iron bioavailability, and longer term biological effects such as growth, remains mostly unknown.

To shed light on the role of EPS on phytoplankton iron limitation, we studied EPS excreted by two Southern Ocean phytoplankton (*E. huxleyi* and *Phaeocystis antarctica*) cultures and one Antarctic sea-ice bacterium (*Pseudoalteromonas* sp.) culture as well as EPS isolated from the surface waters of the Sub-Antarctic Zone (SAZ) at the time of a phytoplankton bloom dominated by coccolithophorids during the 2010 austral summer. We compared the effect of these substances to model iron binding organic compounds on iron bioavailability, photo-physiology, and phytoplankton growth using the diatom *Chaetoceros simplex*. In addition, the impact of a previously characterized bacterial EPS (*Pseudoalteromonas* sp. isolated from the Southern Ocean, Mancuso Nichols et al., 2004; Hassler et al., 2011b) on iron bioavailability and natural phytoplankton community structure was evaluated from two sites with contrasted nutrient distribution in the Tasman Sea and in the Sub-Antarctic Zone (Table 1, Hassler et al., 2014).

## 2. Methods

### 2.1. EPS isolation

EPS were isolated as per Norman et al. (in press). Algal EPS was isolated from *P. antarctica* (CS 243, Prydz Bay, Antarctica) and *E. huxleyi* (CS 812, Mercury Passage, Tasmania, Australia), maintained axenically in

**Table 1**

Average parameters for samples collected at the depth of the fluorescence maximum ( $F_{max}$ ) in the North Tasman Sea (P1) and the Sub-Antarctic Zone (P3) for incubation experiments during the PINTS voyage. Parameters are dissolved iron (dFe), labile iron (FeLabile) expressed relative to dFe, iron binding ligands (sumL as the sum of all detected ligands; and  $L_1$  as the strong ligand group) and their conditional stability constants with respect to inorganic iron ( $K_{Fe'sumL}$ ). Labile iron is operationally defined by the experimental setup and the electrochemical technique used; it represents the iron concentration in the sample that is exchangeable towards the 10  $\mu$ M TAC added following an overnight equilibration. Dissolved macronutrient concentrations as well as total chlorophyll *a* (Tot Chl *a*) are shown. The phytoplankton community was size fractionated into pico- (0.7–2  $\mu$ m), nano- (2–10  $\mu$ m) and micro-phytoplankton (>10  $\mu$ m) by sequential size filtration and relative contributions were calculated from their respective Chl *a*. <DL = below detection limit, 0.011  $\mu$ M for  $NO_x$ ; ND = not detected. Measurements compared well with those measured from the depth profile (Hassler et al., 2014).

	P1 (165 0 °E, 30 0 °S)	P3 (159 5 °E, 46 2 °S)
Depth of $F_{max}$ (m)	95	25
dFe (nM)	0.03	0.29
Sum of L (nM)	2.24	2.60
Log $K_{Fe'sumL}$	11.62	11.61
$L_1$ (nM)	ND	ND
Log $K_{Fe'L_1}$	ND	ND
FeLabile (%)	58	39
$NO_x$ ( $\mu$ M)	<DL	2.81
Si ( $\mu$ M)	1.02	0.67
$PO_4$ ( $\mu$ M)	0.12	0.35
$F_v/F_m$	0.67	0.55
Tot Chl <i>a</i> ( $\mu$ g $L^{-1}$ )	0.344	0.686
Picophytoplankton (%)	57.7	29.2
Nanophytoplankton (%)	39.8	62.6
Microphytoplankton (%)	2.5	8.2

0.2- $\mu$ m filtered seawater collected in the Sub-Antarctic Zone (SAZ-Sense voyage, RV *Aurora Australis*, 45 3 °S, 153 1 °E, 11th Feb. 2007) and enriched with micronutrients (Fe, Zn, Co = 5 nM, Cu and Ni = 2 nM, Se = 1 nM all buffered by natural ligands present), chelated macronutrients ( $NO_x = 30 \mu$ M,  $PO_4 = 2 \mu$ M, Si = 30  $\mu$ M) and vitamins (as per medium F/20). Axenicity was verified using DAPI stain for *Phaeocystis* and the absence of bacterial fatty acid signature for both strains. Natural EPS was isolated from filtered water sampled at the depth of the fluorescence maximum in the Sub-Antarctic Zone (SAZ, P3, 46 2 °S, 159 5 °E) during the PINTS voyage (RV *Southern Surveyor*, Jan–Feb. 2010, Table 1). The filtrate, containing EPS, was collected into a 25 L clean acid washed carboy to which  $NaN_3$  was added (Sigma) to avoid bacterial growth and kept at 4 °C in the dark until ultrafiltration (Labscale TFF system, 10 kDa MWCO PES membrane, Pellicon R XL 50 Cassette, Millipore). The  $NaN_3$  was lost during ultrafiltration and rinsing steps; 0.14 mg EPS  $L^{-1}$  was recovered from the SAZ sample. The bacterial strains (*Pseudoalteromonas* sp.) were isolated from Antarctic sea ice and Southern Ocean seawater and phenotypically characterized as described previously (Mancuso Nichols et al., 2005). Culture filtrates were diafiltered with 10 volumes of sterile Milli-Q™ water and concentrated at room temperature (VivaFlow 200 ultrafiltration unit, PES membrane, 100 kDa MWCO, Sartorius). The concentrated (50 mL) EPS solutions were frozen overnight and the water was removed by freeze-drying (DynaVac). The iron concentration associated with each EPS was analyzed by inductively coupled mass spectrometry (see Norman et al., for experimental detail); it was 393, 2274, 7112, 5267 nmol iron  $g^{-1}$  EPS for EPS from sea ice bacteria, SAZ bloom, *P. antarctica* and *E. huxleyi*, respectively.

### 2.2. EPS impact on cultured and natural phytoplankton

To assess the biological impact of iron associated with EPS compared to inorganic iron, phytoplankton originating from an axenic culture and two natural sites were used. An Antarctic diatom was chosen as good model phytoplankton strain as diatom as present world-wide,

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