Short Note: Extracellular Export and Consumption of Glucose in Antarctic Sea Ice

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Abstract

Glucose microsensors were used to measure extracellular glucose exudation and consumption in a sea ice algal community. Glucose export increased with increasing irradiance between 15 and 512 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. This export correlated with declining F_vF_m values and increasing NPQ values, implying that glucose export resulted from exposure to above optimal irradiances. Glucose concentrations in samples treated with DCMU to block photosynthesis, declined at all irradiances. Bacterial consumption of glucose was between 6% and 34% of extracellular export per hour.

Introduction

Polar sea ice contains high biomass, diverse microbial communities, that make a major contribution to the annual primary productivity of perennially ice-covered polar seas (Arrigo 2014). Biomass is typically dominated by diatom communities although other protists, bacteria, Archea and viruses are also abundant (McMinn et al. 2020). Even though temperatures and under ice irradiances are low, productivity rates are often high and comparable to productive communities elsewhere (Lizotte 2001; McMinn et al. 2012; van Leeuwe et al. 2018).

Extracellular carbohydrate production (EPS) is widespread in sea ice microbial communities, being produced by both algal cells and bacteria (Ugalde et al. 2014). One class of carbohydrates, extracellular polymeric substances (EPS), has been shown to have multiple roles that include cell motility, substrate attachment and protection (Underwood and Kromkamp 1999; Staats et al. 2000; Cook et al. 2007). Much of this EPS is comprised of polysaccharides, uronic acids, and sulphated sugars (Underwood and Paterson 2003; Bellinger et al. 2009; Oakes et al. 2010; Rivaró et al. 2021). Microalgal dissolved organic carbon (DOC) production and export is a fundamental characteristic that underpins the functioning of microbial food webs. After export from the microalgae, the DOC is rapidly taken up by both heterotrophic bacteria and the microalgae. Extracellular release of DOC from microalgae is mostly by either cell lysis, resulting from grazing or viral attack, or photosynthetic overflow, i.e., where photosynthetic carbon fixation exceeds what is necessary for macromolecular synthesis and the resulting excess photosynthate is released directly into the water column (Smith and Underwood 2000). The availability of an organic substrate is essential for heterotrophic bacterial growth and the effective functioning of the microbial loop (Azam et al. 1983). There is consequently often a close and coupled association between autotrophic producers, such as diatoms, and bacterial consumers (Fenchel 2008). The composition of DOC exudates from microalgae is dominated by glucose, which often comprises more than 70% of the total (Hama and Yanagi 2001; Haas and Wild 2010, Underwood et al. 2010). The causes and drivers of extracellular DOC export (through photosynthetic overflow) are not well understood, but any process that leads to a reduction in growth, such as nutrient limitation or excessive light (Staats et al. 1999; Underwood and Paterson 2003; Cook et al. 2004, 2007) is likely to contribute. Extracellular DOC exudation in culture, for instance, is mostly associated with the low nutrient, stationary phase of growth rather the exponential phase (Myklesstad 1977).

While a range of organic carbon compound categories (e.g. lipids, proteins, carbohydrates etc) have previously been quantified in the EPS of sea ice (Palmisano and Sullivan 1985; Aslam et al. 2012; Ugalde et al. 2016), glucose itself was first identified as an important component of sea ice habitats by Underwood et al. (2010). Extracellular glucose production has since been documented by Aslam et al. (2016). Here we examine extracellular glucose exudation and consumption in sea ice exposed to a light gradient. We hypothesise that excessive irradiance will lead to an increase in glucose exudation.

Methods

Three replicate sea ice cores were collected from Cape Evans, Antarctica, by SIPRE corer on 12th November 2019. The bottom 5 mm of each core was shaved into pre-chilled filtered sea water and stored in the dark at $\sim 0^{\circ}\text{C}$ for 20 minutes for the cells to acclimate.

Subsamples (5 ml) from each ice core were placed in 3 ml cuvettes and exposed to light levels of 15, 68, 227 and $512 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for three to five minutes for the glucose concentrations to be measured. This time period was considered sufficient to measure the change in glucose concentration over time (i.e., extracellular glucose production/consumption) but insufficient for algal and bacterial biomasses to change. Glucose measurements were made every 1 s. The control sample, which contained no sea ice algae, was also measured at each irradiance. DCMU (concentration 20 mM) was added to an additional set of samples from the same cores and measured at the same range of irradiances. All incubations and measurements occurred at 0°C .

Glucose measurements

A glucose biosensor (Pinnacle Technology Lawrence KS, USA) was used to measure net glucose exudation following McMinn and Lee (2018). This biosensor uses the oxidase enzyme to reduce the glucose molecules and produce hydrogen peroxide as a by-product. The hydrogen peroxide is reduced on a platinum electrode to produce electrons that are then detected on a Ag/AgCl reference electrode (McMinn and Lee 2018). The biosensor was modified from those commercially available at Pinnacle Technology Inc. to increase their sensitivity, which was required for the biosensors to be able to measure glucose exudation by ice algae in this study. As relatively high glucose concentrations are required for detection ($> 2\text{--}5 \mu\text{M L}^{-1}$), it was important to use relatively high biomass sea ice samples. The output of each biosensor (picoamps) was calibrated against a standard curve (D-glucose standard, Sigma Aldrich, St. Louis, MO, USA), comprised of glucose solutions at concentrations of 0.125, 0.25, 0.375, 1.375, 2.375 mM L^{-1} . This standard curve was used to convert the raw millivolt signal of the biosensors to actual glucose concentrations. The biosensors have a 90% response time of $\sim 4 \text{ s}$ (McMinn and Lee 2018).

The glucose biosensor was mounted onto a motorised micromanipulator (Unisense A/S, Aarhus, Denmark) and connected to a field multimeter (Unisense, A/S, Aarhus, Denmark); the raw data were converted into mg glucose L^{-1} using the relationship from the standard curve.

To obtain total glucose exudation, the glucose consumption in the dark (the control) was added to the glucose production in the light. Glucose consumption in the dark represents bacterial uptake of glucose. The exudation rate was measured by determining the change in glucose concentration over two minutes.

Exudation rates were normalized to chlorophyll *a* (chl *a*) concentrations and time (mg glucose [mg chl *a*]⁻¹ hr⁻¹). Chlorophyll *a* concentration of each replicate was measured by fluorometer (Turner 10AU, KA), using the acidification method.

Pulse amplitude modulated fluorometry (PAM)

Chlorophyll *a* fluorescence of photosystem II was measured using a pulse amplitude-modulated fluorometer (Water-PAM, Walz, Effeltrich, Germany) with an internal actinic light source centred on 660 nm. All samples were dark-acclimated for 20 min prior to measurement.

A more complete description of the PAM methods used is described in Kennedy et al. (2020).

Fluorescent induction curves, which were used to examine the effect of increasing irradiance on PSII reaction centre kinetics and energy dissipation mechanisms, were obtained under software control (WinControl 3; Walz). Cells were first exposed to a brief pulse of far-red illumination (wavelength > 680 nm) for 5 s to oxidize PSI and gain an estimate of F_0 . Induction curves were initiated by first determining F_v/F_m by a saturation pulse, followed

by a delay of 30 seconds before actinic illumination (either 15, 68, 227 or 516 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) was turned on. Additional saturation pulses were performed at the onset of actinic illumination and every 20 seconds thereafter until the cessation of actinic light four minutes later. The final saturation pulse at four minutes was used to determine the final F_v/F_m value and non photochemical quenching (NPQ). NPQ represents the fraction of heat that is dissipated via regulated photoprotective mechanisms such as the xanthophyll cycle and is derived from:

$$\text{NPQ} = (F_m - F_m') / F_m' \text{ (Genty et al. 1989).}$$

Statistics

Pearson correlations were performed to observe relationships between glucose exudation, F_v/F_m and NPQ using the software package Rstudio (RStudio team (2021), version 1.4.1106). Data were checked for normality using the Shapiro-Wilk test.

Results

Glucose measurements

Independent glucose measurements were made at each of the four light treatments (15, 68, 227, 512 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). At each irradiance, additional samples were used to measure a control (no added

sea ice algae) and samples treated with DCMU, to inhibit photosynthetic activity. At all light levels, glucose concentrations in the control samples were beneath the level of detection. Glucose concentrations across all other samples ranged from 0.012–0.167 mg L⁻¹. Net extracellular glucose productivity rates were 0.115 ± 0.193, 0.152 ± 0.004, 2.427 ± 2.068 and 1.791 ± 0.233 mg glucose (mg chl *a*)⁻¹ hr⁻¹ at 15, 68, 227 and 512 μmol photons m⁻² s⁻¹, respectively (Table 1). Samples treated with DCMU experienced a drop in glucose concentrations, presumably mostly due to bacterial consumption. Glucose consumption rates were 0.051 ± 0.021, 0.144 ± 0.0593 and 0.138 ± 0.0001 mg glucose (mg chl *a*)⁻¹ hr⁻¹ at 68, 227 and 512 μmol photons m⁻² s⁻¹, respectively (Table 1, Fig. 1). Electrode response in the 15 μmol photons m⁻² s⁻¹ sample was unstable and so no consumption rate could be measured. Bacterial consumption was responsible for ~ 34%, 6% and 8% of extracellular glucose production per hour, equivalent to glucose pool turnover times of 2.9, 17 and 13 h⁻¹ at 68, 227 and 512 μmol photons m⁻² s⁻¹, respectively, although the large standard deviations for some of the measurements means these values are indicative only. There was a moderate correlation between extracellular glucose export and bacterial uptake ($r^2 = 0.68$, $p = 0.001$)

Pulse Amplitude Modulation (PAM) measurements

F_v/F_m values at the beginning of the inductions curves at the four irradiances were between 0.331 ± 0.156 and 0.476 ± 0.019 (Table 2). However, at the end, after four minutes actinic light exposure these had dropped from 0.303 ± 0.082 at 15 μmol photons m⁻² s⁻¹ to 0.206 ± 0.120, 0.025 ± 0.031, and 0.109 ± 0.018 at 68, 227 and 512 μmol photons m⁻² s⁻¹, respectively. Over the same time interval, NPQ values increased from 0.253 ± 0.027 at 15 μmol photons m⁻² s⁻¹ to 0.430 ± 0.298, 0.696 ± 0.432, and 0.779 ± 0.293 at 68, 227 and 512 μmol photons m⁻² s⁻¹, respectively (Table 2). There was a moderate correlation between extracellular glucose export and % decline in F_v/F_m ($r^2 = 0.75$, $p = 0.00026$) and a weak correlation between extracellular glucose export and maximum NPQ ($r^2 = 0.39$, $p = 0.031$).

Table 1
Extracellular glucose export and consumption. Light (μmol photons m⁻² s⁻¹); export rate (mg glucose (mg chl *a*)⁻¹ hr⁻¹); bacterial uptake rate (mg glucose (mg chl *a*)⁻¹ hr⁻¹)

Light	Export rate	Bacterial uptake	Turnover time
15	0.115 ± 0.193		
68	0.152 ± 0.004	0.051 ± 0.021	2.9
227	2.427 ± 2.068	0.144 ± 0.059	17
512	1.791 ± 0.233	0.138 ± 0.0001	13

Table 2

Photosynthetic parameters. Irradiance ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$); F_v/F_m (start); F_v/F_m (dimensionless ratio) at the start of the induction curve after 30 min dark acclimation. F_v/F_m (end); F_v/F_m (dimensionless ratio) at the end of the 4 min induction curve; % decline: % drop in F_v/F_m between the beginning and end of the induction curve, NPQ; non photosynthetic quenching (dimensionless).

Irradiance	F_v/F_m (start)	F_v/F_m (end)	% decline	NPQ
15	0.476 ± 0.019	0.303 ± 0.082	36	0.253 ± 0.027
68	0.331 ± 0.156	0.206 ± 0.120	38	0.355 ± 0.248
227	0.371 ± 0.077	0.025 ± 0.031	93	0.432 ± 0.020
512	0.471 ± 0.035	0.019 ± 0.018	96	0.779 ± 0.293

Discussion

In this study, net extracellular glucose export in a sea ice community from McMurdo Sound increased with increasing irradiance, with a step increase between 68 and 227 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. DOC production and exudation in marine biofilms, including sea ice, has been well documented and has usually been found to be associated with high light levels and/or nutrient limitation (Staats et al. 1999; Underwood and Paterson 2003; Cook et al. 2004, 2007; Ugalde et al. 2014; Underwood et al. 2013). Most studies have shown that sea ice communities reach light saturation, i.e., have E_k values, at irradiances of less than 200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, with inhibition starting at $< 300 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (McMinn et al. 2012). Similarly, *in situ* communities exposed to ambient irradiances have F_v/F_m values of < 0.1 , compared with night-time values of > 0.4 (McMinn et al. 2003). Most studies actually show E_k values less than 100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (McMinn et al. 2002, 2003, 2007, 2010). In this study also, there was a sharp drop in final F_v/F_m and an increase in NPQ values, between irradiances of 68 and 227 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. NPQ are protective mechanisms that are upregulated to divert energy from excess irradiance to prevent cellular damage. In this study NPQ increased at all irradiances but was much greater after exposure to higher irradiances. There is a clear correlation between these increased NPQ values and glucose export. As under-ice inorganic nutrient levels in spring in McMurdo Sound are consistently high (Arrigo 2014, Cummings et al. 2019). This relationship implies that it is likely that the above optimal irradiances alone caused the increase in DOC exudation (glucose) in the sea ice community.

The addition of DCMU to the samples inhibited electron flow between PSII and PSI, thus preventing carbon fixation and the production of glucose. The subsequent drop in dissolved glucose concentrations thus potentially resulted from both biotic and abiotic processes. However, in similar experiments with benthic microalgae, McMinn and Lee (2018) treated samples in the dark with antibiotics to inhibit bacterial activity. These samples showed no significant decline in glucose concentrations with time, indicating that abiotic breakdown of glucose was occurring at a much slower rate. Thus, bacterial

consumption was responsible for most of the drop in glucose concentrations in the ice algae samples treated with DCMU. Glucose turnover times were between 2.9 and 17 hours, rates consistent with open marine and freshwater turnover times elsewhere (Alonso-Saez et al. 2012; Skoog et al. 2002; Bunte and Simon 1999).

This study, together with that of McMinn and Lee (2017), has demonstrated the importance of low molecular weight DOC (in particular glucose) in the microbial dynamics of substate-bound communities. It has also demonstrated that microsenors can be reliably used to measure microalgal exudation and bacterial uptake rates of glucose in these communities. Microsenors are well suited to these systems as biomass levels and DOC concentrations are often many times higher than in open water, which overcomes microsenor sensitivity issues.

Declarations

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Availability of data: all data is presented within the manuscript

Author Contributions

AMcM, FK and AM conceived and designed the experiments. FK conducted the experiments. AMcM and FK analysed the data and AMcM wrote the manuscript. All authors read and approved the manuscript.

Ethical standards: no ethics issues involved

Consent to participate/publish: each author has approved their inclusion in this publication

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Figures

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Figure 1

Glucose consumption rates were 0.051 ± 0.021 , 0.144 ± 0.0593 and 0.138 ± 0.0001 mg glucose (mg chl a)⁻¹ hr⁻¹ at 68, 227 and 512 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, respectively