Marine Chemistry 187 (2016) 16–24

Contents lists available at ScienceDirect

Marine Chemistry

journal homepage: www.elsevier.com/locate/marchem

Silicon content of individual cells of *Synechococcus* from the North Atlantic Ocean

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ARTICLE INFO

Article history:
Received 22 May 2016
Received in revised form 13 September 2016
Accepted 18 October 2016
Available online 21 October 2016

Keywords:
Silicon
Synechococcus
Synchrotron X-ray fluorescence
SXRF
Elemental quotas

ABSTRACT

The widely distributed marine cyanobacterium *Synechococcus* is thought to exert an influence on the marine silicon (Si) cycle through its high cellular Si relative to organic content. There are few measurements of Si in natural populations of *Synechococcus*, however, and the degree to which *Synechococcus* from various oligotrophic field sites and depths accumulate the element is unknown. We used synchrotron x-ray fluorescence to measure Si quotas in individual *Synechococcus* cells collected during three cruises in the western North Atlantic Ocean in the summer and fall, focusing on cells from the surface mixed layer (SML; <10 m) and the deep chlorophyll maximum (DCM). Individual cell quotas varied widely, from 1 to 4700 amol Si cell^{-1}, though the middle 50% of quotas ranged between 17 and 119 amol Si cell^{-1}. Mean station-specific quotas exhibited an even narrower range of 31–72 amol Si cell^{-1}. No significant differences in Si quotas were observed across cruises or among stations, and no effect of ambient silicic acid concentration on quotas was observed within the narrow range of silicic acid concentrations encountered (0.6–1.3 μM). Despite this small range in ambient silicic acid, cells collected from the SML had an average of two-fold more Si than cells collected from the DCM. Differences in Si content with depth may be related to observed differences in the dominant *Synechococcus* clades between the SML and DCM habitats, determined by *petB* gene sequencing.

1. Introduction

Photoautotrophic picocyanobacteria from the genus *Synechococcus* are found in nearly all ocean surface waters and have been shown to accumulate Si. Surprisingly, they can attain biomass-normalized cellular Si:S and Si:P mole ratios that are, on average, up to 50% of those in siliceous diatoms (Baines et al., 2012). *Synechococcus* are most abundant in nutrient-rich upwelling systems and in coastal and estuarine environments (Partensky et al. 1999), but they are also found in oligotrophic open-ocean systems like the Costa Rica Dome at abundances as high as 10^5 to 10^6 mL^{-1} (Saito et al., 2005; Stukel et al., 2013). Persistent accumulation of Si by *Synechococcus* presents an additional pathway for Si uptake that is not controlled by diatoms. Furthermore, recent analyses have suggested that ≥75% of the euphotic zone biogenic silica (bSi) pool is "detrital" (i.e. not associated with living diatoms) (Krause et al., 2010; Marquez, 2015). Contributions by *Synechococcus* would have been systematically included in this detrital pool instead of within picoplankton size cells. Therefore, the direct consideration and inclusion of *Synechococcus* biomass may be important to accurately modeling the Si cycle in ocean regions where *Synechococcus* biomass is significantly higher than diatoms.

Since the discovery of Si accumulation by marine *Synechococcus* by Baines et al. (2012), other studies have investigated this organism's potentially outsized effects on other elemental cycles and on vertical C export. Tang et al. (2014) demonstrated accumulation of Mg and Si in the protein- and carbohydrate-rich extracellular polymeric substances (EPS) of degrading *Synechococcus* cultures and in water column micro-aggregates near Bermuda. Deng et al. (2015b) further showed that *Synechococcus* detritus scavenges C from exopolymers and dissolved organic matter, forming rapidly sinking (440–660 m d^{-1}) aggregates that may export 2–3 times more C than in *Synechococcus* cells alone. Recent results from the *Tara* oceans expedition have implicated *Synechococcus* and its phages as having central, strategic roles in C export networks of the oligotrophic gyres (Guidi et al., 2016). Scavenging

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http://dx.doi.org/10.1016/j.marchem.2016.10.003
0304-4203/

and vertical export by *Synechococcus* biomass thus appears to be more significant to a range of elemental budgets than previously thought (Lomas et al., 2010).

The *Synechococcus* genus has wide temperature, nutrient, and light tolerances due to its considerable genetic diversity. Numerous clades have been defined using genetic techniques, and many clades show preferences for specific marine niches (Tai and Palenik, 2009) though little is known about the degree to which natural field populations accumulate Si. With the abundance and geographic range of *Synechococcus* expected to grow in response to global climate change (Flombaum et al., 2013), their influence on the marine Si cycle and other elemental cycles may also grow. The observed expansion of the oligotrophic gyres (Polovina et al., 2008) and decadal declines in open-ocean diatom biomass (Krause et al., 2009) underscore the importance of understanding the interactions of natural field *Synechococcus* populations with Si. How do typical *Synechococcus* cellular Si quotas (i.e. mol Si per cell) vary across environments, seasons, and within various marine niches (e.g. surface mixed layer [SML], deep chlorophyll maximum [DCM])? We investigated these questions through synchrotron x-ray fluorescence (SXRF)-based examination of single-cell elemental quotas in field-collected *Synechococcus* during the summer and early fall in the western North Atlantic.

2. Methods

2.1. Bottle sampling: BATS and BVAL cruises

Whole seawater samples were collected during Bermuda Atlantic Time Series (BATS) cruises in July, 2012 (BATS cruise 283) and October, 2012 (BATS 286) and during a BATS validation cruise in September–October, 2013 (BVAL 48; stn. 2) on the R/V Atlantic Explorer (Fig. 1). The BVAL cruise then continued south towards Puerto Rico, occupying several stations from which only SML samples were analyzed. Seawater was collected from the SML (5–10 m sampling depth) and the depth of the subsurface DCM (75–88 m sampling depth; determined for each cast via in situ fluorometry) using the ship's CTD rosette. All reported parameters were measured on water collected from the same bottles. For single-cell SXRF mapping, whole seawater was preserved with glutaraldehyde at a final concentration of 0.25% and cells were deposited on Au electron microscopy grids using centrifugation at 1700 rcf for 30 min. Grids were briefly rinsed with deionized water to remove residual salt. Microscopy was used to locate orange-fluorescing, single *Synechococcus* targets for later SXRF analyses using an excitation wavelength of 480 nm and a long-pass emission filter. Cell size (ca. 1 μm) and visual morphology (ovoid shape) were also used to distinguish *Synechococcus* cells from other auto-fluorescing cell types.

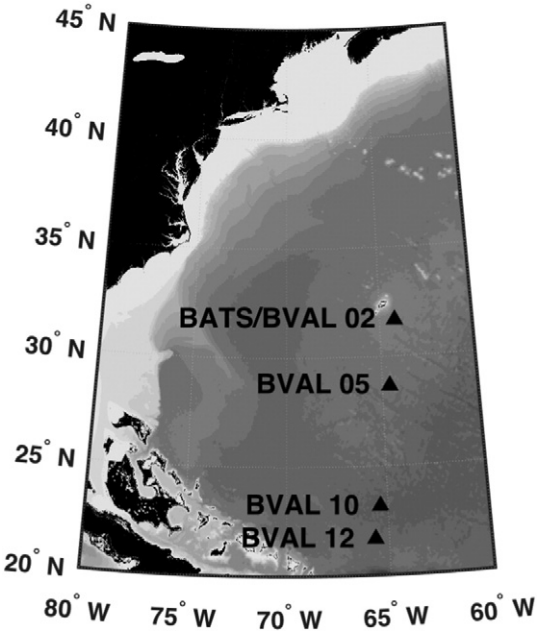

Fig. 1. Locations of major sampling stations in the western Atlantic.

A wide range of values were observed for several environmental parameters in part due to local sampling site variability within cruises (Table 2). This variation is likely due to a combination of vessel drift and advection of eddy features past the ship's position. Samples analyzed from BATS cruise 283 (July 2012) were collected during two casts on sequential days. *Synechococcus* abundances and small bSi (0.4–3 μm size-fraction) were notably higher at the SML than at the DCM on cast 1, while cast 3 exhibited similar values for both parameters at both depths. SXRF samples from BATS cruise 286 (Oct 2012) were also collected during two casts on sequential days, with significantly higher DCM *Synechococcus* abundances on cast 3. During the BVAL cruise, *Synechococcus* cell abundances in the SML generally increased towards the south, though compared to the BATS cruises, *Synechococcus* cell abundances and bSi were both generally lower and exhibited smaller ranges during this cruise.

2.2. SXRF methodology

Samples were analyzed at the Advanced Photon Source beamline 2-ID-E with an incident energy of 10 keV using protocols similar to those previously described (Twining et al., 2011, 2003). Focused beam size was up to ~ 0.4 μm in the vertical and ~0.8 μm in the horizontal. Step sizes of 0.1 μm were used to over-sample target cells and background areas. The pixels covering the cell were averaged, and the mean signal from neighboring cell-free areas was subtracted from this average. The background-corrected concentration was then multiplied by the area of the analyzed cell region, resulting in a mol cell^{-1} measurement referred to herein as the cell quota.

Four detector elements collected SXRF spectra simultaneously at each pixel, and fluorescence data from each detector was quantified independently using NBS Standard 1833 and an AXO thin-film SXRF standard. Lighter elements (Si, P) that are absent in the standards were fit using a least squares linear regression through the $\log_{10}$ of expected quantum yield for the elements present in the standards, as detailed in Núñez-Milland et al. (2010).

For the picocyanobacteria analyzed in this study, slight differences in geometric orientation of the detector elements relative to the sample, combined with the shallow 15° observation angle to the sample grid (which has vertical topography significantly larger than many cells) occasionally created inconsistencies in the concentrations measured by the four detector elements. Detectors generally agreed on quantifications of high atomic mass (Z) elements (e.g. Fe, Ni), but occasionally two or three detectors would measure anomalously low quantifications for the low-Z elements (Si, P, S). We suspect that in these cases, fluorescent x-rays emitted by target cells were being shaded by the Au grid bars or by larger nearby cells, with the effect more noticeable for low-Z elements which emit lower-energy fluorescent x-rays that are more prone to absorption. This effect was detector-specific, implicating the geometry of the sample grid, cell region of interest, and detector position. To locate and compensate for this effect, for each mapped region of interest (ROI) and detector we plotted the measured elemental quotas (normalized to the highest detector) versus Z using MATLAB (The Mathworks Inc., Natick, MA, USA). Shaded detectors were identified as those that exhibited a roughly linear decrease in relative quantification at lower Z. Data from shaded detectors were excluded in the final elemental analyses and from detection limit and sensitivity determinations.