Algal Research 29 (2018) 36–40

Contents lists available at ScienceDirect

Algal Research

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

The effect of different light regimes on diatom frustule silicon concentration

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ARTICLE INFO

Keywords:
Coscinodiscus granii
Light wavelength
Light intensity
Cellular Si content
Si concentration per surface

ABSTRACT

Significant changes in frustule morphology of *Coscinodiscus granii* induced by different light regimes have previously been observed, but whether these changes are companied by similar changes in cellular silicon concentration or are solely due to reorganization is unknown. The influence of six different light regimes, blue (B), green (G), yellow (Y), red-orange (RO), red (R) and white (W), at two intensities (100 and 300 μmol photons $m^{-2} s^{-1}$) on cellular silicon content was therefore assessed for *C. granii*. Both the cellular Si content and the Si concentration per surface area were higher at 300 than at 100 μmol photons $m^{-2} s^{-1}$ for all tested wavelengths, except Y light. At the same light intensity, cells grown at B light had the highest cellular Si content (except W at 300 μmol photons $m^{-2} s^{-1}$) and Si concentration per surface area. At R, OR, G, B and W light, high intensity led to higher cellular Si content although the mean frustule size was smaller (opposite for Y light). As the higher Si content was not due to a larger cell size, we hypothesize that the effect is due to changes in valve thickness. The underlying adaptive significance of this phenomenon might be that at low irradiance (100 μmol photons $m^{-2} s^{-1}$), diatom buoyancy is modulated by accumulating less silicon in the cells, as the less silicified cells may have lower sinking rate and thus remain longer at the higher light in the upper part of the water column, which could partly contribute to the ecological success of the diatom.

1. Introduction

Diatoms are one of the dominant groups of photosynthetic organisms on a global scale, accounting for 20–40% of the oceanic primary production [1,2]. A key and unique characteristic of diatoms is their box-like silica wall (the frustule) surrounding the cell, making them an easily recognizable algal group in both living and fossil assemblages.

Amorphous silica is an essential component of the frustule [1,3], and studies of diatom silica morphogenesis [4–6] indicate that cellular silicon content within a species varies considerably throughout the cell cycle; it is higher during the G2 and M periods (between cytokinesis and daughter cell separation) [7]. Higher salinities lead to a decrease in silicification due to osmotic stress [8], and other abiotic factors, such as silicate concentration in the medium, pH, nutrient limitation and temperature may also affect the cellular Si content [8,9]. Although light is one of the most important abiotic factors for microalgal growth and metabolism [10,11], only a few studies have looked at the effect of different light intensities on cellular Si content in diatoms [7,12,13]. Further, although longer wavelengths of light are rapidly absorbed by the water and blue light penetrates deeper in the ocean than the other wavelengths [14], to the best of our knowledge, only one study has focused on the link between light regimes (blue and red light at 85 μmol photons $m^{-2} s^{-1}$) and cellular silicon content [15], and no studies have tested the effect of different light regimes throughout the visible light spectrum at different intensities on the cellular Si content.

Coscinodiscus granii is a typical centric marine diatom with large and flat valves [1], which has been explored for its optical properties and the corresponding optical applications [16,17]. Besides, previous studies showed that when *C. granii* was exposed to different wavelengths at two different intensities (100 or 300 μmol photons $m^{-2} s^{-1}$), morphological changes in the frustule silica nanostructure were observed [18,19], but it was not reported whether the frustule Si concentration changed or was just reorganized.

In this study, five different monochromatic light regimes throughout the visible spectrum - B, G, Y, RO and R- were compared to broad-spectered W light at two photon flux densities (100 and 300 μmol photons $m^{-2} s^{-1}$) with regard to frustule Si concentrations of *Coscinodiscus granii* L.F. Gough. The study offer new insight into potential ecological implications of differences in cellular Si contents.

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https://doi.org/10.1016/j.algal.2017.11.014
Received 21 June 2017; Received in revised form 11 November 2017; Accepted 11 November 2017
Available online 20 November 2017

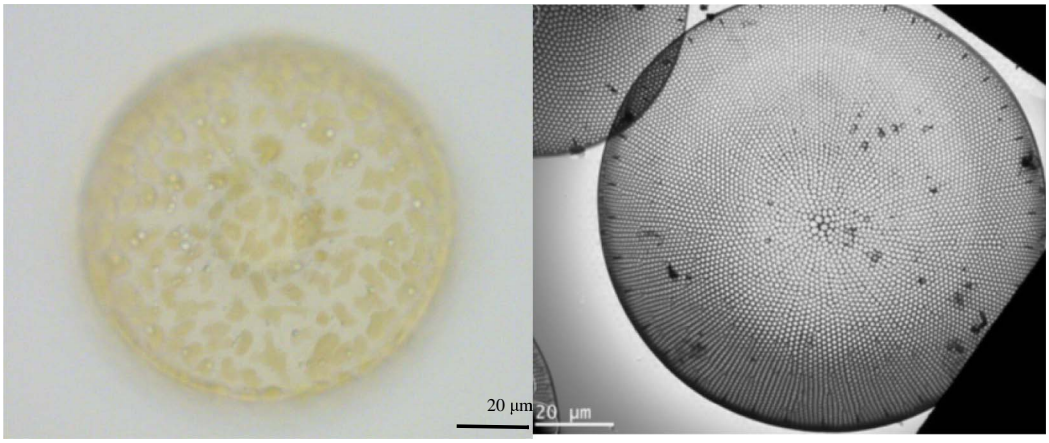

Fig. 1. Living *C. granii* cell under microscope (A), transmission electron micrograph (TEM) image of a valve of *C. granii* (B).

2. Materials and methods

2.1. Cultures

Coscinodiscus granii (strain K-1834, Fig. 1) used in this study was obtained from Scandinavian Culture Collection of Algae & Protozoa (now a part of NIVA culture collection of algae, https://niva-cca.no/). The strain was kept at 20 °C and 100 μmol photons $m^{-2} s^{-1}$ (Panasonic FL40ss Enw/37, Japan) with a light: dark cycle of 16 h: 8 h without aeration or shaking. The growth medium used was L1 medium [20] with a salinity of 30 based on autoclaved seawater.

2.2. Experimental setup and operation

Light-emitting diodes (LEDs) with six different light wavelengths (listed in Table 1): B light (455 nm), G light (528 nm), Y (590 nm), RO (617 nm) and R (654 nm) and the control (W light, full visible spectrum) (OSRAM, UK) were employed as illumination. For each wavelength, two irradiance levels, 300 μmol photons $m^{-2} s^{-1}$ (300) and 100 μmol photons $m^{-2} s^{-1}$ (100) were compared. Light was measured with a universal light meter (ULM-500, WALZ, Germany). The experiments were performed in incubators (MLR-352, Panasonic, Japan) without aeration or shaking at a stable temperature (20 ± 1 °C). After one week of acclimation at each combination of wavelength and light intensity, triplicate Falcon flasks with 50 ml of L1 medium at an initial pH of 8.0 were inoculated with *C. granii* cells from exponentially growing cultures. The initial cell concentration was 30 ± 2 cells/ml (counted in a Sedgewick Rafter Counting Chamber). Cells were counted every day, and after ten days, when the cultures had reached stationary phase, the cells were counted again (300 μmol photons $m^{-2} s^{-1}$: around 1000 cells/ml for all the different light wavelengths comprising R, RO, Y, G, B and W light; 100 μmol photons $m^{-2} s^{-1}$: around 900 cells/ml for cells grown at R, B, G and W light, around 700 cells/ml for RO light and around 400 cells/ml for Y light), and harvested for further analysis.

Table 1
The description of the LEDs used in this study.

LED emission color	Dominant wavelength (nm)	Model number
Blue (B)	455	ILH-GD01-DEBL-SC201
Green (G)	528	ILH-GD01-TRGR-SC201
Yellow (Y)	590	ILH-GD01-YELL-SC201
Red orange (RO)	617	ILH-GD01-RDOR-SC201
Red (R)	654	ILH-GD01-HYRE-SC201
White (W)	Full visible spectrum	ILH-GD01-ULWH-SC201

2.3. Diatom cellular Si content and frustule morphological parameters

Total cellular silicon content was determined as described by Paasche [13]. Briefly, cells in a 25 ml subsample were filtered through a 25 mm nuclepore polycarbonate filter with a 0.6 μm pore size (Whatman, Germany), rinsed with 2 ml MilliQ water and immediately placed in a 50 ml polypropylene tube. After adding 18 ml of 0.5% Na_2CO_3 solution, the tube was placed in a water bath (OBN 18, Holm & Halby, Denmark) at 85 °C for 2 h for hydrolysis. After cooling down to room temperature, a few drops of 0.5 N HCl were added to adjust the pH until the titration point of methyl orange (pH 3–4), and subsequently MilliQ water was added to reach 25 ml. A blank sample was made by using an unused nuclepore polycarbonate filter through the same procedure as aforementioned. Afterwards, all the cellular silicon content was transformed into the reactive silicate concentration, which was determined based on the molybdate method [21] with an UV/Vis spectrophotometer (Hitachi U-2001, Japan). The detailed processes were as follows: (1) mixing 10 ml of molybdate solution with 25 ml aforementioned prepared samples; (2) allowing the mixture stand for 10 min; (3) adding reducing reagent (mixing 100 ml of metol-sulphite solution with 60 ml of oxalic acid solution, and then adding 60 ml 50% sulphuric acid solution, finally mixing distilled water to a volume of 300 ml) rapidly to the volume of exactly 50 ml and mixing immediately; (4) measuring with spectrophotometer at a wavelength of 810 nm after allowing the solution obtained from (3) to stand for 2–3 h.

The frustule surface ($A = \pi r^2$) was calculated based on previously published morphological parameters on the same strain [18]. Si concentration per surface area was calculated by dividing cellar Si content by mean frustule surface.

3. Results and discussion

The diatom frustule is composed of organic materials and amorphous hydrated silica with the general formula $[Si_n\ O_{2n-(nx/2)}(OH)_{nx}]$, with $x \leq 4$ [3,22]. Silica is regarded as the most abundant metal element in the frustule [8]. Nearly all of the cellular Si is in the frustule, with a small fraction found in cytoplasm and organelles, especially the mitochondria and chloroplasts [1,12,13]. Further, previous research indicates that the Si measurement method used in this study represents mostly frustule silicon [13]. Cellular silicon content may vary substantially within a diatom species, even up to 4-fold [3,12,13], and our measurements are within this range (Fig. 2).

Variation in both size and thickness of the frustule may lead to differences in cellular Si content [3]. Therefore we compared our data to data from Su et al. [18] and calculated the silicon content pr. cell surface area as well as pr. cell. Overall, higher cellular Si contents were found at 300 than at 100, with the exception of cells grown under Y