Research Letter

Effect of Low pH on Carbohydrate Production by a Marine Planktonic Diatom (*Chaetoceros muelleri*)

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Rising carbon dioxide (CO_2) concentrations in the atmosphere due to human activity are causing the surface ocean to become more acidic. Diatoms play a pivotal role in biogeochemical cycling and ecosystem function in the ocean. pH affected the quantum efficiency of photosystem II and carbohydrate metabolism in a planktonic diatom (*Chaetoceros muelleri*), representative of a widely distributed genus. In batch cultures grown at low pH, the proportion of total carbohydrate stored within the cells decreased and more dissolved carbohydrates were exuded from the cells into the surrounding medium. Changes in productivity and the way in which diatoms allocate carbon into carbohydrates may affect ecosystem function and the efficiency of the biological carbon pump in a low pH ocean.

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1. Introduction

Rising carbon dioxide (CO_2) concentrations in the atmosphere due to human activity are causing the ocean to become more acidic [1]. The pH of the upper ocean has decreased from a preindustrial value of 8.2 to approximately 8.1 today [1]. It is estimated that the pH of ocean surface waters will be 7.9 by the end of this century and 7.4 by the end of the millennium [2]. Despite the global biogeochemical significance of primary production by diatoms, little is known about how they will respond to the decreasing pH of the ocean. The carbohydrate glucan is the primary storage compound in diatoms for carbon fixed during photosynthesis. Moreover, diatoms exude a proportion of their photosynthate into the surrounding medium as extracellular carbohydrates. The objective of this research was to determine how seawater pH affects growth, photosynthesis, and carbohydrate metabolism in a common diatom.

2. Materials and Methods

Chaetoceros muelleri (Lemmermann) (CCMP 1316) was grown at pH 6.8, 7.4, 7.9, and 8.2. Cultures grown at pH 6.8

represented an extreme beyond the current predictions for future ocean pH [2]. Batch cultures were grown in artificial seawater salt base [3] to which nutrients, trace metals, and vitamins were added as an f/2 enrichment [4]. Cultures were grown at 20°C under a photosynthetic photon flux density of $50 \mu\text{mol m}^{-2}\text{s}^{-1}$, with a 14-hour daily light period. pH was maintained within the cultures using a biological buffer (25 mM HEPES (Sigma-Aldrich, St. Louis, Mo, USA)) and by daily titration with a small amount ($<100 \mu\text{L}$) of 1 M HCl.

Growth each day was followed in the cultures using in vivo chlorophyll fluorescence measured in a small sample ($200 \mu\text{L}$) using a spectrofluorometer plate reader (Molecular Devices, Sunnyvale, Calif, USA).

The six replicate cultures grown at each pH were extensively sampled during exponential growth phase on day 5. Samples were taken for cell counts [4] and chlorophyll *a* concentrations [5]. The maximum quantum efficiency of photosystem II (F_v/F_m) in *Chaetoceros muelleri* was measured in dark adapted (2 hours) cultures using a pulse amplitude-modulated (PAM) fluorometer (Heinz Walz, Effeltrich, Germany) [6]. Carbohydrates were measured using the phenol-sulfuric acid assay calibrated with D-glucose [7]. Fractionation and extraction of the different carbohydrate

FIGURE 1: Effect of medium pH on photosynthesis and carbohydrate metabolism in *Chaetoceros muelleri*. Samples taken after 5 days growth in batch culture. (a) Maximum quantum efficiency of photosystem II (F_v/F_m). (b) Total, cell and dissolved extracellular carbohydrate concentrations. (c) Cell-associated carbohydrates. (d) Proportion of carbohydrates in the dissolved extracellular and cell storage pools. (e) Extracellular carbohydrate pools. (f) Concentration of transparent exopolymer particles (TEP). Bars show mean + standard deviation ($n = 6$ replicate cultures).

TABLE 1: *Chaetoceros muelleri* biomass and growth. Actual pH was the pH measured over the course of 6 days in culture. Growth rate derived from changes in in vivo chlorophyll fluorescence between days 2 and 5. Biomass is expressed as diatom concentration (cells mL⁻¹) and chlorophyll *a* (chl. *a*) concentration (μg L⁻¹). These data were used to calculate chl. *a* content per cell (fg cell⁻¹) on day 5. Values are means ± standard deviation (*n* = 6 replicate cultures).

pH	Actual pH	Growth rate (μ')	Doubling time (days)	Cells mL ⁻¹	chl. <i>a</i> (μg L ⁻¹)	chl. <i>a</i> (fg cell ⁻¹)
6.8	6.82 ± 0.04	0.19 ± 0.01	1.55 ± 0.05	1.36 × 10 ⁶ ± 6.89 × 10 ⁴	518 ± 89	378 ± 50
7.4	7.42 ± 0.04	0.24 ± 0.02	1.27 ± 0.10	1.83 × 10 ⁶ ± 9.26 × 10 ⁴	687 ± 141	375 ± 76
7.9	7.93 ± 0.04	0.25 ± 0.01	1.22 ± 0.03	1.84 × 10 ⁶ ± 8.84 × 10 ⁴	644 ± 80	351 ± 45
8.2	8.23 ± 0.05	0.25 ± 0.01	1.20 ± 0.07	1.65 × 10 ⁶ ± 8.08 × 10 ⁴	637 ± 68	388 ± 55

pools were carried out using established methods [8, 9]. Transparent exopolymer particles (TEP) were quantified according to [10].

3. Results and Discussion

Acid titration was used to maintain the desired pH [11], which tended to drift upward due to the removal of aqueous CO₂ during photosynthesis. Future work should aim to decrease the pH by bubbling CO₂-enriched air through seawater [12], which both reduces pH and increases the dissolved inorganic carbon (DIC) concentration. This simulates the effect of rising atmospheric *p*CO₂ and provides control over DIC concentration.

Photosynthesis was affected by pH as determined by *F_v/F_m* (see Figure 1(a)). *F_v/F_m* was inversely correlated with seawater pH (*r* = 0.90, *P* < .001, *n* = 24). These data show that the photosynthetic efficiency of *C. muelleri* decreases as the environment surrounding the cells becomes more acidic. Therefore, less energy was available to the cells to fix carbon at relatively low pH. Growth rate was not affected by pH between 7.4 and 8.2; however, growth rate at pH 6.8 was significantly lower (analysis of variance; *F*_{3,20} = 24.2, *P* < .001) (see Table 1).

Total carbohydrate was the greatest in cultures grown at the preindustrial pH of 8.2 (see Figure 1(b)). There was a significant decrease in total (*F*_{3,20} = 21.6, *P* < .001) and cell (*F*_{3,20} = 22.7, *P* < .001) carbohydrate as the diatoms were grown at lower pH. In contrast, there was a significant increase (Kruskal-Wallis ANOVA; *H* = 18.6, 3 degrees of freedom, *P* < .001) in dissolved extracellular carbohydrates with decreasing pH. The ratio of cell carbohydrate to dissolved extracellular carbohydrate was 2.60 ± 0.26 (mean ± SD) at pH 8.2; decreasing to 1.99 ± 0.41 at pH 7.9, and 1.24 ± 0.41 at pH 7.4 (*F*_{3,20} = 30.3, *P* < .001). There was a significant difference (*F*_{3,19} = 6.55, *P* = .003) in non-storage cell carbohydrates at different growth pH (see Figure 1(c)).

As pH decreased, a decreasing proportion of the total carbohydrate was stored within the cells as glucan, and a greater proportion of the total carbohydrate was exuded from the cells (see Figure 1(d)). There was a significant inverse correlation between pH and the ratio of dissolved extracellular carbohydrate to glucan (*r* = 0.91, *n* = 23, *P* < .001). The increase in dissolved extracellular carbohydrate concentrations with decreasing pH was mainly in the form of low molecular weight carbohydrates rather

than exopolymers (see Figure 1(e)). This is supported by the measurements of transparent exopolymer particle (TEP) concentrations within the cultures, which were not significantly different at different pH (see Figure 1(f)).

The shift in carbon allocation to different pools by diatoms at low pH may have implications for the ecosystem function, the efficiency of the biological carbon pump, and the resulting sequestration of CO₂ in the deep ocean [13]. Less carbohydrate stored within the cells, and a greater production of low-molecular-weight dissolved extracellular carbohydrates at low pH, will result in less particulate organic carbon in the water column, potentially reducing the efficiency of the biological carbon pump during diatom blooms. The reduction in *F_v/F_m* and the increase in the proportion of carbohydrates exuded from the cells suggest that low pH affects membrane function in *Chaetoceros muelleri*. Thylakoid membrane integrity is vital to maintain photosynthetic capability and the plasmalemma controls exchange between the intra- and extracellular environments. Further work, including larger-scale mesocosm experiments in situ, will be needed to test these hypotheses.

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