Journal of Experimental Marine Biology and Ecology 481 (2016) 9–14

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

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

CO_2-dependent carbon isotope fractionation in dinoflagellates relates to their inorganic carbon fluxes

Mirja Hoins [a,b,*], Tim Eberlein [b], Dedmer B. Van de Waal [c], Appy Sluijs [a], Gert-Jan Reichart [a,d], Björn Rost [b]

[a] Department of Earth Sciences, Faculty of Geosciences, Utrecht University, Budapestlaan 4, 3584 CD Utrecht, The Netherlands
[b] Marine Biogeosciences, Alfred Wegener Institute, Helmholtz Centre for Polar- and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany
[c] Department of Aquatic Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Droevendaalsesteeg 10, 6708 PB Wageningen, The Netherlands
[d] Royal Netherlands Institute for Sea Research (NIOZ), Landsdiep 4, 1797 SZ 't Horntje, Texel, The Netherlands

ARTICLE INFO

Article history:
Received 12 September 2015
Received in revised form 3 April 2016
Accepted 4 April 2016
Available online 30 April 2016

Keywords:
CCM
CO_2 uptake
HCO_3^- uptake
Leakage

ABSTRACT

Carbon isotope fractionation (ε_p) between the inorganic carbon source and organic matter has been proposed to be a function of pCO_2. To understand the CO_2-dependency of ε_p and species-specific differences therein, inorganic carbon fluxes in the four dinoflagellate species *Alexandrium fundyense, Scrippsiella trochoidea, Gonyaulax spinifera* and *Protoceratium reticulatum* have been measured by means of membrane-inlet mass spectrometry. In-vivo assays were carried out at different CO_2 concentrations, representing a range of pCO_2 from 180 to 1200 μatm. The relative bicarbonate contribution (i.e. the ratio of bicarbonate uptake to total inorganic carbon uptake) and leakage (i.e. the ratio of CO_2 efflux to total inorganic carbon uptake) varied from 0.2 to 0.5 and 0.4 to 0.7, respectively, and differed significantly between species. These ratios were fed into a single-compartment model, and ε_p values were calculated and compared to carbon isotope fractionation measured under the same conditions. For all investigated species, modeled and measured ε_p values were comparable (*A. fundyense, S. trochoidea, P. reticulatum*) and/or showed similar trends with pCO_2 (*A. fundyense, G. spinifera, P. reticulatum*). Offsets are attributed to biases in inorganic flux measurements, an overestimated fractionation factor for the CO_2-fixing enzyme RubisCO, or the fact that intracellular inorganic carbon fluxes were not taken into account in the model. This study demonstrates that CO_2-dependency in ε_p can largely be explained by the inorganic carbon fluxes of the individual dinoflagellates.

1. Introduction

During photosynthetic carbon fixation, the lighter carbon isotope ^{12}C is preferred over the heavier carbon isotope ^{13}C, thereby causing carbon isotope fractionation (ε_p) between the inorganic carbon (C_i) source and the organic carbon. Values for ε_p of marine phytoplankton have been shown to be CO_2-sensitive (e.g. Degens et al., 1968), and thus were discussed to serve as a proxy for past CO_2 concentrations (Jasper and Hayes, 1990; Pagani, 2014; Van de Waal et al., 2013; Hoins et al., 2015). Large species-specific differences in ε_p have been described, which are yet poorly understood (e.g. Hinga et al., 1994; Burkhardt et al., 1999). Moreover, irrespective of the phytoplankton species investigated, most of these studies have solely described the relationship between ε_p and CO_2, and only few have investigated the underlying physiological processes. Such mechanistic understanding is, however, needed to identify the reasons of the CO_2-dependency of ε_p.

Carbon isotope fractionation of phytoplankton is primarily driven by the enzyme ribulose-1,5-bisphosphate Carboxylase/Oxygenase (RubisCO), which is responsible for the fixation of CO_2 into organic compounds. The intrinsic fractionation associated with RubisCO (ε_f) has been estimated to range between ~22 and 30‰ (e.g. Roeske and O'Leary, 1984; Guy et al., 1993; Scott et al., 2007), even though a recent study obtained values as low as 11‰ for the RubisCO of the coccolithophore *Emiliania huxleyi* (Boller et al., 2011). While RubisCO principally sets the upper limit of fractionation, other processes strongly determine the degree to which RubisCO can express its fractionation (Sharkey and Berry, 1985; Burkhardt et al., 1999; Rost et al., 2002). First, there is leakage, i.e. the amount of CO_2 diffusing out of the cell in relation to C_i uptake. With higher leakage, the intracellular C_i pool is 'refreshed', thereby preventing accumulation of ^{13}C and allowing RubisCO to approach its upper fractionation values. Second, the relative

Abbreviations: C_i, inorganic carbon; CCM, CO_2-concentrating mechanism; Chl-*a*, Chlorophyll-*a*; ε_p, carbon isotope fractionation; $\varepsilon_{p\text{-}meas}$, measured carbon isotope fractionation; $\varepsilon_{p\text{-}mod}$, modeled carbon isotope fractionation; ε_s, equilibrium fractionation between CO_2 and HCO_3^-; ε_f, kinetic fractionation associated with the CO_2 fixation of RubisCO; L_{CO2}, ratio of CO_2 efflux relative to total C_i uptake; DIC, dissolved inorganic carbon; HCO_3^-, bicarbonate; R_{HCO3}, ratio of HCO_3^- to total C_i uptake; RubisCO, ribulose-1,5-bisphosphate Carboxylase/Oxygenase; CA, carbonic anhydrase; TA, total alkalinity.

* Corresponding author at: Department of Earth Sciences, Faculty of Geosciences, Utrecht University, Budapestlaan 4, 3584 CD Utrecht, The Netherlands.
E-mail addresses: mhoins@awi.de (M. Hoins), tim.eberlein@awi.de (T. Eberlein), D.vandeWaal@nioo.knaw.nl (D.B. Van de Waal), a.sluijs@uu.nl (A. Sluijs), g.j.reichart@uu.nl (G.-J. Reichart), bjoern.rost@awi.de (B. Rost).

http://dx.doi.org/10.1016/j.jembe.2016.04.001

contribution of bicarbonate (HCO_3^-) to total C_i uptake plays a role, as HCO_3^- is enriched in ^{13}C by ~10‰ relative to CO_2 (Mook et al., 1974). An increasing HCO_3^- contribution thus lowers ε_p. The enzyme carbonic anhydrase (CA), which accelerates the otherwise slow interconversion between CO_2 and HCO_3^-, can also influence ε_p under certain conditions, e.g. by influencing leakage as well as the relative HCO_3^- contribution. All these processes play a role in the CO_2-concentrating mechanisms (CCMs) of phytoplankton. Assessing the mode of CCMs may therefore help to understand the reasons for CO_2-dependent changes in ε_p and species-specific differences therein.

Dinoflagellates are cosmopolitan unicellular algae that occur in many different environments, including eutrophic coastal regions and oligotrophic open oceans. In this study, we investigated whether the CO_2-dependency of ε_p, which was found in the dinoflagellate species *Alexandrium fundyense, Gonyaulax spinifera*, *Protoceratium reticulatum* and *Scrippsiella trochoidea* (Burkhardt et al., 1999; Hoins et al., 2015), can be explained by changes in their C_i fluxes. Characteristics of CCMs in the tested species, including their CA activities and C_i fluxes, were measured by means of membrane-inlet mass spectrometry (MIMS). Results were fed into a single-compartment model that considers cellular leakage, the relative HCO_3^- contribution as well as the carbon isotope fractionation of RubisCO (Sharkey and Berry, 1985; Burkhardt et al., 1999). The calculated carbon fractionation ($\varepsilon_{p\text{-}mod}$) was then compared to the measured carbon fractionation ($\varepsilon_{p\text{-}meas}$).

2. Material and methods

2.1. Incubations

Cultures of the dinoflagellate species *A. fundyense* (formerly *Alexandrium tamarense* strain Alex5; John et al., 2014), *S. trochoidea* (strain GeoB267; culture collection of the University of Bremen), *G. spinifera* (strain CCMP 409) and *P. reticulatum* (strain CCMP 1889) were grown in 0.2 μm filtered North Sea water (salinity 34), which was enriched with 100 μmol L^{-1} nitrate and 6.25 μmol L^{-1} phosphate. Metals and vitamins were added according to f/2 medium (Guillard and Ryther, 1962), except for $FeCl_3$ (1.9 μmol L^{-1}), H_2SeO_3 (10 nmol L^{-1}) and $NiCl_2$ (6.3 nmol L^{-1}) that were added according to K medium (Keller et al., 1987). Each of the strains was grown in 2.4 L air-tight borosilicate bottles at 15 °C and 250 ± 25 μmol photons m^{-2} s^{-1} at a 16:8 h light:dark cycle. Bottles were placed on roller tables in order to avoid sedimentation.

Dissolved CO_2 concentrations ranged from ~5–50 μmol L^{-1} and were reached by pre-aerating culture medium with air containing 180, 380, 800 and 1200 μatm pCO_2. The carbonate chemistry was calculated based on pH and total alkalinity (TA), using the program CO2sys (Pierrot et al., 2006). pH values were measured using a WTW 3110 pH meter equipped with a SenTix 41 Plus pH electrode (WTW, Weilheim, Germany), which was calibrated prior to measurements to the National Bureau of Standards (NBS) scale. An automated TitroLine burette system (SI Analytics, Mainz, Germany) was used to determine TA. Dissolved inorganic carbon (DIC) was determined colorimetrically using a QuAAtro autoanalyser (Seal Analytical, Mequon, USA). For more details on the carbonate chemistry in the acclimations, please refer to Eberlein et al. (2014) for *A. fundyense* and *S. trochoidea* and to Hoins et al. (2015) for *G. spinifera* and *P. reticulatum*.

To determine ε_p values, the isotopic composition of the organic material was measured using an Automated Nitrogen Carbon Analyser mass spectrometer (ANCA-SL 20–20, SerCon Ltd., Crewe, UK), and the isotopic composition of the DIC in growth medium was measured using a GasBench-II coupled to a Thermo Delta-V advantage isotope ratio mass spectrometer (see Hoins et al., 2015 for details on isotope analysis). Prior to assays, cells were acclimated to the different CO_2 concentrations for at least 7 generations (i.e. >21 days). To prevent changes in the carbonate chemistry, i.e. keeping drawdown of DIC <3%, incubations were terminated at low cell densities (<400 cells mL^{-1}).

2.2. MIMS assays

A custom-made membrane-inlet mass spectrometer (MIMS; Isoprime, GV Instruments, Manchester, UK; see Rost et al., 2007 for details) was used to determine CA activities and C_i fluxes of *A. fundyense* and *S. trochoidea* acclimated to four different pCO_2 (i.e. 180, 380, 800 and 1200 μatm; Eberlein et al., 2014), and of *G. spinifera* and *P. reticulatum* acclimated to a low and high pCO_2 (i.e. 180 and 800 μatm). Assays were performed in an 8 mL temperature-controlled cuvette, equipped with a stirrer. Assay tests over ~1 h confirmed that conditions during the assay do not cause physiological stress (i.e. no decline in O_2 production rates), and subsequent microscopic inspection did not reveal any visual effects on cell morphologies. Prior to the measurements, acclimated cells were concentrated using a 10 μm membrane filter (Millipore, Billerica, MA) by gentle vacuum filtration (<200 mbar) and stepwise transferred into C_i-free medium buffered with a 4-(2-hydroxylethyl)-1-piperazine-ethanesulfonic acid (50 $mmol^{-1}$ HEPES) solution at 15 ± 0.3 °C and a pH of 8.0 ± 0.1. Chlorophyll *a* (Chl-*a*) concentrations were determined fluorometrically by using a TD-700 Fluorometer (Turner Designs, Sunnyvale, CA, USA) and ranged between 0.15 and 1.70 μg mL^{-1} during the assays.

To quantify activities of extracellular CA (eCA), the ^{18}O depletion rate of doubly labeled $^{13}C^{18}O_2$ in seawater was determined by measuring the transient changes in $^{13}C^{18}O^{18}O$ (m/z = 49), $^{13}C^{18}O^{16}O$ (m/z = 47) and $^{13}C^{16}O^{16}O$ (m/z = 45) in the dark, following the approach of Silvermann (1982). If cells possess eCA, exchange rates of ^{18}O are accelerated relative to the spontaneous rate. To monitor the spontaneous rate, $NaH^{13}C^{18}O_3$ label was injected to the cuvette, waiting until the m/z = 49 signal reached a steady-state decline. This rate was then compared to the steady-state decline after cells were added. Following Badger and Price (1989), eCA activity is expressed as percentage decrease in ^{18}O-atom fraction upon the addition of cells, normalized to Chl-*a*. Consequently, 100 units (U) correspond to a doubling in the rate of interconversion between CO_2 and HCO_3^- per μg Chl-*a*.

Photosynthetic O_2 and C_i fluxes were determined following Badger et al. (1994). Making use of the chemical disequilibrium, this approach estimates CO_2 and HCO_3^- fluxes during steady-state photosynthesis. It is based on the simultaneous measurements of O_2 and CO_2 concentrations during consecutive light and dark intervals with increasing amounts of DIC. Oxygen fluxes in the dark and light are converted into C_i fluxes by applying a respiratory quotient of 1.0 and a photosynthetic quotient of 1.1 (Burkhardt et al., 2001; Rost et al., 2003). The light intensity in the cuvette was adjusted to the acclimation conditions (i.e. 250 ± 25 μmol photons m^{-2} s^{-1}). Net CO_2 uptake was calculated from the steady-state decline in CO_2 concentration at the end of the light period, corrected for the interconversion between CO_2 and HCO_3^-. The uptake of HCO_3^- was calculated by subtracting net CO_2 uptake from net C_i uptake, and the CO_2 efflux from the cells was estimated from the initial slope after turning off the light. Rate constants k_1 and k_2 were determined based on temperature, salinity and pH (Zeebe and Wolf-Gladrow, 2001; Schulz et al., 2006), yielding mean values of 0.9241 (±0.0506) min^{-1} and 0.0085 (±0.0008) min^{-1}, respectively. To eliminate any eCA activity, a prerequisite to apply the rate constants, we added dextran-bound sulfonamide (DBS; 50 μmol L^{-1}) to the cuvette. For more details on the calculations, please refer to Badger et al. (1994) and Schulz et al. (2007).

2.3. Single-compartment model

To calculate $\varepsilon_{p\text{-}mod}$, results for the relative HCO_3^- contribution and leakage were fed into a single-compartment model after Sharkey and