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NOTE

Relationship between aqueous CO_2 concentrations and stable carbon isotope discrimination in the diatoms *Chaetoceros calcitrans* and *Ditylum brightwellii*

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ABSTRACT: Recent work has focused on a possible relationship between marine organic $\delta^{13}C$ and the concentration of carbon dioxide dissolved in seawater [CO_2(aq)]. This relationship is based on the general assumption that diffusive CO_2 uptake is the main pathway of photosynthetic carbon acquisition by phytoplankton. This study found an inverse linear relationship between [CO_2(aq)] and organic $\delta^{13}C$ in the marine diatoms *Chaetoceros calcitrans* and *Ditylum brightwellii*. However, the relationship was not a function of diffusive CO_2 use by these diatoms, which have previously been shown to be HCO_3^- users. Our findings underline the importance of understanding the mechanisms of phytoplankton carbon acquisition in interpreting carbon isotope data.

KEY WORDS: Organic $\delta^{13}C$ · Carbon dioxide · Bicarbonate · *Chaetoceros calcitrans* · *Ditylum brightwellii*

Today, after more than 30 yr of research, there is still no satisfactory explanation for the wide variation of $\delta^{13}C$ values found in marine phytoplankton ($\delta^{13}C_{org}$). Factors such as temperature (Sackett et al. 1965, Wong & Sackett 1978, Hinga et al. 1994), salinity (Wong & Sackett 1978, Hinga et al. 1994), pH (Hinga et al. 1994, Thompson & Calvert 1994), growth rate (Takahashi et al. 1991, Laws et al. 1995, Korb et al. 1996), cell size (Fry & Wainwright 1991, Korb et al. 1996) and intracellular carbon demand (Rau et al. 1992, François et al. 1993, Laws et al. 1995), among others, have been reported to determine the isotopic discrimination seen in phytoplankton. Recent laboratory and field studies have provided strong evidence that aqueous CO_2 concentration, or [CO_2(aq)], is the major variable determining $\delta^{13}C$ values of marine microalgae (Rau et al. 1992, 1996, François et al. 1993, Goericke et al. 1994). Such a relationship presents the possibility of reconstructing the composition of past atmospheres from the isotopic composition of marine sedimentary records (Freeman & Hayes 1992, Hayes 1993, Rau 1994). However, this association is usually interpreted in terms of inorganic carbon acquisition occurring solely by diffusion of CO_2. In fact, there are few reports of marine phytoplankton that are unable to utilize HCO_3^-. HCO_3^- use and/or uptake other than by CO_2 diffusion may serve to weaken the correlation between organic $\delta^{13}C$ and [CO_2(aq)] (Raven 1993).

In this paper, further evidence for a relationship between [CO_2(aq)] and isotopic composition is presented and the results are discussed in view of the inorganic carbon sources used for photosynthesis.

Continuous cultures of the marine diatoms *Chaetoceros calcitrans* Paulsen and *Ditylum brightwellii* (West) Grun. were grown as described in Korb et al. (1996) at a photon flux density of 60 µmol m^{-2} s^{-1}. At steady state, the specific growth rate of *C. calcitrans* was 1.30 d^{-1} and 0.5 d^{-1} for *D. brightwellii*. Aeration was provided at a rate of 2 dm^3 min^{-1} from air drawn from outside the constant temperature room in which the cultures were situated. Dissolved inorganic carbon (DIC), pH and biomass were kept relatively constant (Table 1) and checks were made on these parameters approximately every 2 to 3 d. DIC was measured using an infra-red gas analyser, acid stripping technique (Johnston & Raven 1986). Cell counts were made using a Coulter counter (Model ZM, Coulter Electronics) and chlorophyll *a* was determined spectrophotometrically using the equations of Jeffrey & Humphrey (1975). Nutrients were not limiting for growth. For stable carbon isotope analysis, approximately 150 cm^3 of culture was harvested as described by Korb et al. (1996). The $\delta^{13}C$ of organic carbon was measured on a VG SIRA Series II isotope ratio mass spectrometer. At the time that this work was performed, we did not have a suitable method for measuring source $\delta^{13}C$ values, therefore results are expressed as isotopic composition rather than discrimination.

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Table 1. Continuous culture conditions of *Chaetoceros calcitrans* and *Ditylum brightwellii* over a period of 30 d ($n \geq 10$, ± SD of the mean)

Species	Biomass (µg chl *a* cm^{-3})	Cell density (cells cm^{-3})	pH	[DIC] (mol m^{-3})	Organic $\delta^{13}C$ (‰)
C. calcitrans	0.24 (± 0.07)	1.6×10^6	8.35 (± 0.10)	2.0 (± 0.13)	−20.25 to −27.96
D. brightwellii	0.25 (± 0.08)	1.9×10^4	8.20 (± 0.18)	2.24 (±0.16)	−23.65 to −27.73

Over a period of 30 d, organic $\delta^{13}C$ values of *Chaetoceros calcitrans* ranged from −20.25 to −27.96‰ and for *Ditylum brightwellii*, −23.65 to −27.73‰. Fluctuations in organic $\delta^{13}C$ values were due to uncontrolled variations in the air supply to the cultures. There was no significant correlation (all p values >0.05) between the small scale changes in cell density, biomass, pH or DIC concentrations to account for variations in organic $\delta^{13}C$ values. [CO_2(aq)] was determined from pH and [DIC] using the equations of Goyet & Poisson (1989) and was found to be significantly ($p < 0.05$) negatively correlated to isotopic composition (Fig. 1). Furthermore, when continuous cultures of *C. calcitrans* were aerated from cylinders of compressed air containing a constant CO_2 source, isotopic composition was stabilized to a mean value of −20.39‰, with a range from −20 to −22‰.

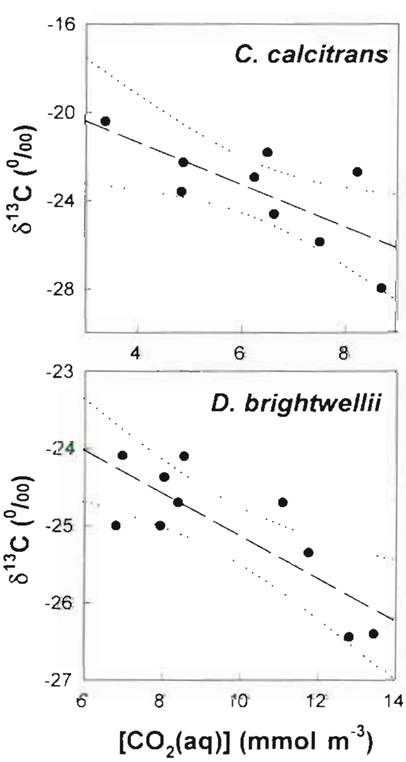

Fig. 1. Relationship between diatom organic $\delta^{13}C$ values and CO_2 concentrations (aqueous). Regression lines are fitted to the data by the equations $\delta^{13}C = -17.49 - 0.96[CO_2(aq)]$ for *Chaetoceros calcitrans* (r = 0.72) and $\delta^{13}C = -22.35 - 0.28[CO_2(aq)]$ for *Ditylum brightwellii* (r = 0.81). Dotted lines indicate 95% confidence intervals

The results presented in this study support the hypothesis that photosynthetic carbon isotope fractionation is a function of [CO_2(aq)] whereby higher CO_2 concentrations favour increasing levels of discrimination against the heavier isotope ^{13}C. Laws et al. (1995) reported similar findings with continuous cultures of *Phaeodactylum tricornutum*, a species with the characteristics of a highly effective HCO_3^- user. In addition, Korb et al. (1997) demonstrated the ability of *Chaetoceros calcitrans* and *Ditylum brightwellii* to use both HCO_3^- and CO_2 as sources of inorganic carbon for photosynthesis. The mechanism by which small scale fluctuations of aqueous CO_2 concentration should influence $\delta^{13}C$ values of diatoms using bicarbonate is unclear, especially in view of the short time scales of the $\delta^{13}C$ and [CO_2] variations relative to the generation time of the diatoms and dilution of the $\delta^{13}C$ appropriate to earlier growth conditions. In this study, cultures were sufficiently well aerated to provide a continual re-supply of CO_2 into the medium and the isotopic species should be in equilibrium. Under such conditions it is possible that the carbon demand of the diatoms could be met by passive CO_2 diffusion. Isotopic discrimination values (Δ) exceeding 20‰ relative to CO_2 are indicative of diffusive CO_2 use (Raven et al. 1995). Assuming that the source CO_2 in our cultures had a value similar to the −7‰ for dissolved CO_2 in seawater (Mook et al. 1974) and using the calculations of Raven & Farquhar (1990), the isotopic discrimination value of *C. calcitrans* is 13.27‰ (organic $\delta^{13}C$ value of −20‰). Such a value is considerably lower than that of isolated, *in vitro* RUBISCO (~29‰), further suggesting ^{13}C-enriched HCO_3^- use.

It is likely that the overall level of discrimination shown in these diatoms is affected by the intracellular aqueous CO_2 pool being supplied from both isotopically light atmospheric CO_2 and the heavy HCO_3^- pool as well as the assimilation of both of these carbon species. While we are still a long way from fully understanding the factors influencing $\delta^{13}C$ variability of marine microalgae, this study demonstrates that any relationship between [CO_2(aq)] and organic $\delta^{13}C$ is not necessarily due to diffusive CO_2 use by phytoplankton. An understanding of the mechanism of microalgal carbon acquisition is important to aid understanding of organic carbon isotope signatures.

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