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NOTE

A model of carbon isotopic fractionation and active carbon uptake in phytoplankton

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ABSTRACT: The carbon isotopic fractionation of phytoplankton photosynthesis (ε_p) has been interpreted by previous authors as inconsistent with active bicarbonate uptake. This interpretation contradicts the results of numerous physiological studies demonstrating significant active bicarbonate uptake in phytoplankton. Using a simple model of cellular regulation of carbon acquisition we show that an upward curvature of ε_p as a function of the ratio of growth rate to carbon dioxide concentration does not exclude active bicarbonate uptake. Our model describes adequately published carbon isotope data for cyanobacteria, diatoms, and coccolithophores consistent with active bicarbonate uptake.

KEY WORDS: Phytoplankton · Carbon isotopic fractionation · Model · Active carbon uptake · Bicarbonate · Carbon dioxide

The carbon isotopic fractionation of phytoplankton photosynthesis (ε_p) is an important biogeochemical signal used, for example, to estimate ancient carbon dioxide concentrations (e.g. Jasper & Hayes 1990). Unfortunately, many observations contradict the existing ε_p models. Most ε_p models consider only diffusive carbon dioxide (CO_2) transport into the cell (e.g. Laws et al. 1995), which is inconsistent with laboratory and field data that demonstrate active uptake of CO_2 and/or bicarbonate (HCO_3^-)(e.g. Sikes et al. 1980, Tortell et al. 1997, for a review see Raven 1997). Further, these models predict a linear relationship between $\mu/[CO_2]$ (the ratio of growth rate, μ, and carbon dioxide concentration, $[CO_2]$) and ε_p (Francois et al. 1993, Laws et al. 1995) which is contradicted by some observations (e.g. Law et al. 1997). Models that incorporate a cellular regulation of active carbon uptake neglect the effects of either growth rate or active HCO_3^- uptake on ε_p (Laws et al. 1997, Yoshioka 1997, Popp et al. 1998).

Based on a model interpretation, Laws et al. (1997) and Popp et al. (1998) concluded that the upward curvature of ε_p with increasing $\mu/[CO_2]$ (i.e. an increasing positive deviation from a linear relationship) which they observed is inconsistent with HCO_3^- uptake. However, independent experiments demonstrate HCO_3^- uptake for several of the very species they investigated (e.g. Sikes et al. 1980, Colman & Rotadore 1995). It is important to resolve this discrepancy, since phytoplankton unable to take up HCO_3^- may be CO_2-limited, even if they actively transport CO_2 (Riebesell et al. 1993). Here we present a simple model of phytoplankton carbon uptake and isotopic fractionation and show that existing isotope data are consistent with active HCO_3^- uptake.

The model. To model ε_p we use an approximated carbon isotope balance of a phytoplankton cell (Francois et al. 1993), resulting in:

$$\varepsilon_p = \varepsilon_{up} + \theta(\varepsilon_{fix} - \varepsilon_{diff}) \quad (1)$$

In this equation ε_{up}, ε_{fix}, and ε_{diff} represent the fractionation effects of the carbon uptake processes, carbon fixation, and diffusive carbon loss from the cell, respectively, and θ is the ratio of cellular carbon loss to carbon influx. ε_p is well approximated by $\delta^{13}C_{CO_2} - \delta^{13}C_{OM}$, the difference between the isotopic compositions of the external CO_2 and the organic matter pools (Goericke et al. 1994). Note that this stylized model neglects a host of potentially important cellular characteristics such as respiration or cellular compartments.

θ is a function of the diffusive CO_2 influx (equal to $[CO_2] \cdot P \cdot A$, where P denotes the membrane permeability and A the membrane surface area), the cellular carbon demand (equal to $\mu \cdot Q_c$, where Q_c represents the cellular carbon content), as well as the active carbon uptake fluxes. To model the regulation of carbon

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uptake in the simplest way possible, we assume that the cells adjust their active carbon uptake in a constant ratio (γ) to their carbon fixation rate. We neglect the diffusive flux of the charged HCO_3^- molecule across the lipid cell membrane as well as the effects of the diffusive boundary layer. The ratio of carbon loss to carbon influx is then:

$$\theta = \frac{1+(\gamma-1)\frac{\mu Q_c}{[CO_2]PA}}{1+\gamma\frac{\mu Q_c}{[CO_2]PA}} \quad (2)$$

ε_{up} is calculated by an isotopic mass balance of the carbon fluxes into the internal CO_2 pool. We assume the fractionation of the carbon uptake mechanism (ε_t) to be equal to the fractionation by diffusion ($\varepsilon_t = \varepsilon_{diff}$ = 0.7‰; O'Leary 1984). Because we assume zero HCO_3^- efflux, all the HCO_3^- actively taken up has to be completely converted into CO_2. In this situation, the intracellular dehydration shows no isotopic fractionation. Finally, in the case of active HCO_3^- uptake, the substrate for the carbon uptake mechanism has an isotopic composition ($\delta^{13}C_{source}$) which is around 9‰ higher than $\delta^{13}C_{CO_2}$ (Mook et al. 1974). ε_{up} is then:

$$\varepsilon_{up} = \varepsilon_t + \frac{\gamma}{\frac{[CO_2]PA}{\mu Q_c}+\gamma}(\delta^{13}C_{CO_2} - \delta^{13}C_{source}) \quad (3)$$

and ε_p becomes:

$$\varepsilon_p = \varepsilon_t + \frac{\gamma}{\frac{[CO_2]PA}{\mu Q_c}+\gamma}(\delta^{13}C_{CO_2} - \delta^{13}C_{source}) + \frac{1+(\gamma-1)\frac{\mu Q_c}{[CO_2]PA}}{1+\gamma\frac{\mu Q_c}{[CO_2]PA}}(\varepsilon_{fix} - \varepsilon_{diff}) \quad (4)$$

For pure diffusive CO_2 uptake (i.e. $\gamma = 0$) this reduces to the model proposed by Francois et al. (1993) (and to a simplified version of the model of Rau et al. 1996), which both predict ε_p to be a linear function of the variable $\mu/[CO_2]$. The variable $\mu/[CO_2]$, which is proportional to the ratio of carbon demand to maximum diffusive CO_2 influx, effectively quantifies the extent of deficiency of diffusive CO_2 supply for the photosynthetic carbon demand.

We use this model to analyze published data for the microalgae *Phaeodactylum tricornutum*, *Porosira glacialis*, and *Emiliania huxleyi*, and the cyanobacterium *Synechococcus* sp. Our model is capable of fitting other isotope data as well (e.g. Fielding et al. 1998, results not shown) and the discussed data sets are chosen to represent a wide variety of phytoplankton species. To estimate the model parameters, we vary them within reasonable ranges to minimize the mean square model error of $\delta^{13}C_{OM}$ (Table 1). For this study, we assume HCO_3^- as substrate for the carbon uptake mechanism (although the model can of course account for active CO_2 uptake). To simplify the discussion, we express the observations and the model results as $\delta^{13}C_{OM}$, normalized to a $\delta^{13}C_{CO_2}$ of −7.5‰ and a $\delta^{13}C_{HCO_3^-}$ of +1.5‰ (representative of seawater at 15°C; Mook et al. 1974, Goericke 1994).

Results. The model fits (Fig. 1) demonstrate that the model is able to represent the main features of the data rather well. The membrane permeabilities of the microalgae are relatively high (varying between 1.1 and 3.3×10^{-5} ms^{-1}) while the ratios of active HCO_3^- uptake to carbon fixation are relatively low (between 0 and 2.3). The *P. glacialis* data are best described in our model with no active HCO_3^- uptake (e.g. $\gamma = 0$). This may be reasonable, given the low growth rates for *P. glacialis* that range between 0.09 and 0.32 d^{-1}. Alternative explanations cannot be excluded, however, such as a different regulation of active carbon uptake than assumed in our model.

For the microalgae (i.e. all the species except *Synechococcus* sp.), $\delta^{13}C_{OM}$ increases significantly with $\mu/[CO_2]$. At very low $\mu/[CO_2]$ values, the diffusive CO_2 exchange fluxes across the membrane are large compared to the carbon fixation and active carbon uptake fluxes. In this situation, the preference for $^{12}CO_2$ by carbon fixation does not significantly affect the isotopic composition of the internal CO_2 pool, because the gross CO_2 fluxes keep the internal and external CO_2 pools close to isotopic equilibrium. The resulting fractionation effect of photosynthesis is close to the large fractionation by carbon fixation, and $\delta^{13}C_{OM}$ is low. As $\mu/[CO_2]$ increases, the diffusive CO_2 exchange fluxes

Table 1. Model parameters, their allowed ranges, and the root mean square error (RMSE) for the model fits. We use the cell surface areas and cellular carbon contents given by Popp et al. (1998). ε_{fix}: fractionation effect of carbon fixation; *P*: cell membrane permeability; γ: ratio of active carbon uptake to carbon fixation rate

Parameter:	ε_{fix}	*P*	γ	RMSE
Unit:	‰	m s^{-1}	–	‰
Allowed range:	20 – 30[a]	3×10^{-8} – 4×10^{-5}[b]	0 – 7.5[c]	–
E. huxleyi	25.3	1.8×10^{-5}	2.2	0.63
P. tricornutum	26.6	3.3×10^{-5}	2.3	1.2
P. glacialis	23.0	1.1×10^{-5}	0.0	3.1
Synechococcus sp.	30.0	3.0×10^{-8}	7.5	1.2

[a]Adopting the range for ribulose 1,5-bisphosphate carboxylase-oxygenase (Goricke et al. 1994) and assuming neglible β-carboxylation
[b]Gutknecht et al. (1977), Salon et al. (1996)
[c]Range between pure diffusive uptake (i.e. $\gamma = 0$) and the maximum value in *Synechococcus* sp. observed by Tchernov et al. (1997)

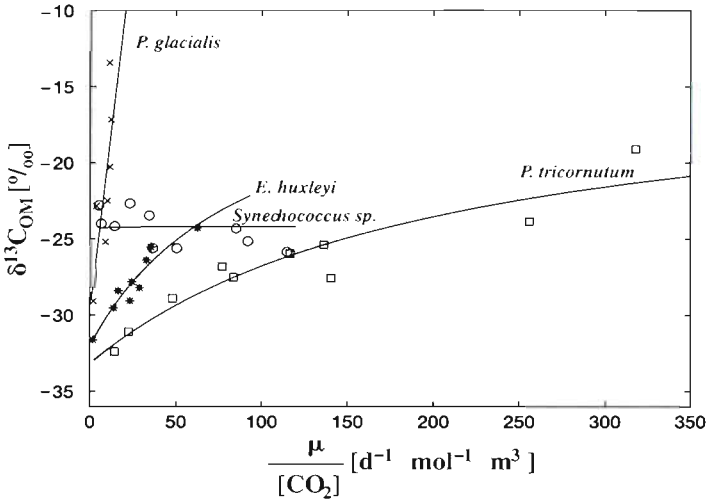

Fig. 1 Comparison of experimental data for *Phaeodactylum tricornutum* (□, Laws et al. 1997), *Synechococcus* sp. (○, Popp et al. 1998), *Emiliania huxleyi* (*, Bidigare et al. 1997), and *Porosira glacialis* (×, Popp et al. 1998) with the model fits (lines). Shown are $\delta^{13}C_{OM}$ data for $\mu/[CO_2]$ values less than 350 d^{-1} mol^{-1} m^3. Model parameters and the root mean square errors of the model fits are given in Table 1

across the membrane decrease relative to the carbon fixation and active carbon uptake fluxes. As a result, the preference for $^{12}CO_2$ by carbon fixation increasingly enriches the internal carbon pool with $^{13}CO_2$, carbon fixation uses more $^{13}CO_2$, and $\delta^{13}C_{OM}$ increases.

For low to intermediate $\mu/[CO_2]$ values the $\delta^{13}C_{OM}$ data for the microalgae may be approximated by a straight line as would be predicted from a pure CO_2 diffusion model, although the net CO_2 diffusion flux may in fact be outwards. For example, for the species *Emiliania huxleyi* at $[CO_2] = 10^{-2}$ mol m^{-3} and $\mu = 0.5$ d^{-1} (resulting in $\mu/[CO_2] = 50$ d^{-1} mol^{-1} m^3) the modeled active HCO_3^- uptake flux is 8.9×10^{-18} mol C cell^{-1} s^{-1} while the net diffusion loss is 4.8×10^{-18} mol C cell^{-1} s^{-1}. The large diffusive CO_2 exchange fluxes (in our example 1.6×10^{-17} mol C cell^{-1} s^{-1} inwards and 2.0×10^{-17} mol C cell^{-1} s^{-1} outwards) dominate the other fluxes and largely determine the isotopic disequilibrium across the membrane. This situation is caused by the relatively high P and low γ. Note that the approximately linear range of $\delta^{13}C_{OM}$ may sometimes be exceeded by oceanic $\mu/[CO_2]$ values (e.g. $\mu/[CO_2] = 260$ d^{-1} mol^{-1} m^3 for $[CO_2] = 8 \times 10^{-3}$ mol m^{-3} and $\mu = 2.1$ d^{-1}) (Eppley 1972, Codispoti et al. 1982).

For the cyanobacterium *Synechococcus* sp., the estimated P is low (3×10^{-8} m s^{-1}, equal to the experimental estimate of Salon et al. 1996), while γ is high (7.5, equal to the maximum value for *Synechococcus* sp. observed by Tchernov et al. 1997 at high light levels). ε_{fix} is estimated as 30‰. A perhaps more realistic fractionation of 21.5‰ (observed for the freshwater cyanobacterium *Anacystis nidulans;* Guy et al. 1993) would be obtained if ε_{up} were around 8‰, or if the cells were taking up predominantly CO_2. However, as long as important model parameters like ε_{up} and ε_{fix} for *Synechococcus* sp. are unknown, the isotope data seem consistent with active HCO_3^- uptake. Note that the model parameters for *Synechococcus* sp. are at the extremes of the imposed ranges (Table 1). Allowing, for example, a lower P would slightly reduce the model error, but violate the experimental estimate of Salon et al. (1996).

$\delta^{13}C_{OM}$ for *Synechococcus* sp. is approximately constant over the range of $\mu/[CO_2]$ of interest. This is explained in our model by the small CO_2 exchange fluxes relative to the other fluxes, caused by the relatively low P and relatively high γ. This results in an approximately constant θ, and $\delta^{13}C_{OM}$.

Discussion. Laws et al. (1997) and Popp et al. (1998) concluded that a downward curvature of $\delta^{13}C_{OM}$ with increasing $\mu/[CO_2]$ excludes active HCO_3^- uptake. In contrast, our analysis shows that the downward curvature of $\delta^{13}C_{OM}$ is consistent with active HCO_3^- uptake. To analyze whether HCO_3^- or CO_2 is actively taken up, Laws et al. (1997) compared the $\delta^{13}C_{OM}$ predicted from a linear extrapolation at low $\mu/[CO_2]$ values with the actual measurements. A downward curvature at higher $\mu/[CO_2]$ values indicates a decrease in $\delta^{13}C_{OM}$ at higher γ. Laws et al. (1997) reasoned that active HCO_3^- uptake would introduce a positive shift in $\delta^{13}C_{OM}$ and is hence inconsistent with the observed downward shift. This reasoning neglects that an increased γ additionally increases the ratio of CO_2 efflux to carbon influx (Eq. 2), relative to the linear extrapolation. This higher ratio of CO_2 efflux to carbon influx acts to decrease $\delta^{13}C_{OM}$ and explains the observed downward curvature—even in the case of active HCO_3^- uptake. In fact, assuming CO_2 instead of HCO_3^- as substrate for the carbon uptake mechanism (i.e. $\delta^{13}C_{source} = -7.5$‰) results in different calibration parameters, but indistinguishable model fits. Experimental data indicate that both HCO_3^- and CO_2 are possible substrates for the carbon uptake mechanism (e.g. Salon et al. 1996, Tchernov et al. 1997). Our results illustrate that the shape of the discussed $\delta^{13}C_{OM}$ data as a function of $\mu/[CO_2]$ is a poor indicator of the carbon species entering the cell.

In conclusion, the discussed isotope data can be described adequately by a simple and plausible model that represents the regulation of active carbon uptake with a single parameter. Neither an approximately linear relationship between $\mu/[CO_2]$ and ε_p nor an upward curvature of ε_p with $\mu/[CO_2]$ can exclude active HCO_3^- or CO_2 uptake. Whether HCO_3^- or CO_2 is actively taken up by a particular microalgae cannot presently be decided on the basis of isotope data (except for the case when $\delta^{13}C_{OM}$ is higher than

$\delta^{13}C_{CO_2}$, indicating that HCO_3^- is a significant carbon source). Available models of carbon isotopic fractionation are insufficiently constrained to distinguish between these possibilities.

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