NOTE

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

Klaus Keller1,*, François M. M. Morel2

1Department of Civil Engineering and Operations Research and 2Department of Geosciences, Princeton University, Princeton, New Jersey 08544, USA

ABSTRACT: The carbon isotopic fractionation of phytoplankton photosynthesis (εp) 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 εp 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 (εp) 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 εp models. Most εp models consider only diffusive carbon dioxide (CO2) transport into the cell (e.g. Laws et al. 1995), which is inconsistent with laboratory and field data that demonstrate active uptake of CO2 and/or bicarbonate (HCO3−) (e.g. Sikes et al. 1980, Tortell et al. 1997, for a review see Raven 1997). Further, these models predict a linear relationship between 14CO2 (the ratio of growth rate, μ, and carbon dioxide concentration, [CO2]) and εp (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 HCO3− uptake on εp (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 εp with increasing 14CO2 (i.e. an increasing positive deviation from a linear relationship) which they observed is inconsistent with HCO3− uptake. However, independent experiments demonstrate HCO3− 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 HCO3− may be CO2− limited, even if they actively transport CO2 (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 HCO3− uptake.

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

\[ \varepsilon_p = \varepsilon_{up} + \theta (\varepsilon_{fix} - \varepsilon_{diff}) \]  

(1)

In this equation εup, εfix, and εdiff 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. εp is well approximated by δ13CO2 − δ13CO2m, the difference between the isotopic compositions of the external CO2 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 CO2 influx (equal to [CO2] · P · A, where P denotes the membrane permeability and A the membrane surface area), the cellular carbon demand (equal to μ · Q, where Q represents the cellular carbon content), as well as the active carbon uptake fluxes. To model the regulation of carbon
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:

$$\theta = \frac{1 + (\gamma - 1) \mu Q_c}{\mu Q_c + \gamma}$$

(2)

$\epsilon_{up}$ 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 ($\epsilon_i$) to be equal to the fractionation by diffusion ($\epsilon_d = \epsilon_{dif} = 0.7\%$; 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). $\epsilon_{up}$ is then:

$$\epsilon_{up} = \epsilon_i + \frac{\gamma}{[CO_2]PA} (\delta^{13}C_{CO_2} - \delta^{13}C_{source})$$

(3)

and $\epsilon_p$ becomes:

$$\epsilon_p = \frac{\gamma}{[CO_2]PA} (\delta^{13}C_{CO_2} - \delta^{13}C_{source})$$

$$+ \frac{1 + (\gamma - 1) \mu Q_c}{\mu Q_c + \gamma} (\epsilon_{inx} - \epsilon_{dif})$$

(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 $\epsilon_p$ to be a linear function of the variable $\gamma/\mu_{\text{CO}_2}$. The variable $\gamma/\mu_{\text{CO}_2}$, 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 $\delta^{13}C_{CO_2}$ (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 $\delta^{13}C_{CO_2}$ 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 $\times 10^{-5}$ m s$^{-1}$) 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.), $\delta^{13}C_{CO_2}$ increases significantly with $\gamma/\mu_{\text{CO}_2}$. At very low $\gamma/\mu_{\text{CO}_2}$ 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 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 $\delta^{13}C_{CO_2}$ is low. As $\gamma/\mu_{\text{CO}_2}$ increases, the diffusive CO$_2$ exchange fluxes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$\epsilon_{up}$</th>
<th>$P$</th>
<th>$\gamma$</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unit:</td>
<td>%</td>
<td>m$^{-2}$ s$^{-1}$</td>
<td>-</td>
<td>%</td>
</tr>
<tr>
<td>Allowed range:</td>
<td>20 $- 30^4$</td>
<td>$3 \times 10^{-8}$ $- 4 \times 10^{-3}$</td>
<td>0 $- 7.5^e$</td>
<td></td>
</tr>
<tr>
<td>E. huxleyi</td>
<td>25.3</td>
<td>1.8 $\times 10^{-3}$</td>
<td>2.2</td>
<td>0.63</td>
</tr>
<tr>
<td>P. tricornutum</td>
<td>26.6</td>
<td>3.3 $\times 10^{-5}$</td>
<td>2.3</td>
<td>1.2</td>
</tr>
<tr>
<td>P. glacialis</td>
<td>23.0</td>
<td>1.1 $\times 10^{-5}$</td>
<td>0.0</td>
<td>3.1</td>
</tr>
<tr>
<td>Synechococcus sp.</td>
<td>30.0</td>
<td>3.0 $\times 10^{-6}$</td>
<td>7.5</td>
<td>1.2</td>
</tr>
</tbody>
</table>

*Adopting the range for ribulose 1,5-bisphosphate carboxylase-oxygenase (Goricke et al. 1994) and assuming negligible P-carboxylation


$^e$ Range between pure diffusive uptake (i.e. $\gamma = 0$) and the maximum value in Synechococcus sp. observed by Tchernov et al. (1997)
across the membrane decrease relative to the carbon fixation and active carbon uptake fluxes. As a result, the preference for $^{13}$CO$_2$ by carbon fixation increasingly enriches the internal carbon pool with $^{13}$CO$_2$, carbon fixation uses more $^{13}$CO$_2$, and $\delta^{13}$CO$_{OM}$ increases.

For low to intermediate $\nu_{[CO_2]}$ values the $\delta^{13}$CO$_{OM}$ 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 $\nu_{[CO_2]} = 50$ d$^{-1}$ mol$^{-1}$ m$^3$) the modeled active HCO$_3^-$ uptake flux is $8.9 \times 10^{-18}$ mol C cell$^{-1}$ s$^{-1}$ while the net diffusional loss is $4.8 \times 10^{-18}$ mol C cell$^{-1}$ s$^{-1}$. The large diffusive CO$_2$ exchange fluxes (in our example $1.6 \times 10^{-17}$ mol C cell$^{-1}$ s$^{-1}$ inwards and $2.0 \times 10^{-17}$ 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 $\gamma$. Note that the approximately linear range of $\delta^{13}$CO$_{OM}$ may sometimes be exceeded by oceanic $\nu_{[CO_2]}$ values (e.g. $\nu_{[CO_2]} = 260$ 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 ($3 \times 10^{-8}$ m s$^{-1}$, equal to the experimental estimate of Salon et al. 1996), while $\gamma$ is high (7.5, equal to the maximum value for Synechococcus sp. observed by Tchernov et al. 1997 at high light levels). $\epsilon_{\text{iaux}}$ 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 $\epsilon_{\text{iaux}}$ were around 8%, or if the cells were taking up predominantly CO$_2$. However, as long as important model parameters like $\epsilon_{\text{iaux}}$ and $\epsilon_p$, 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).

$\delta^{13}$CO$_{OM}$ for Synechococcus sp. is approximately constant over the range of $\nu_{[CO_2]}$ 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 $\gamma$. This results in an approximately constant $\theta$, and $\delta^{13}$CO$_{OM}$.

**Discussion.** Laws et al. (1997) and Popp et al. (1998) concluded that a downward curvature of $\delta^{13}$CO$_{OM}$ with increasing $\nu_{[CO_2]}$ excludes active HCO$_3^-$ uptake. In contrast, our analysis shows that the downward curvature of $\delta^{13}$CO$_{OM}$ 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 $\delta^{13}$CO$_{OM}$ predicted from a linear extrapolation at low $\nu_{[CO_2]}$ values with the actual measurements. A downward curvature at higher $\nu_{[CO_2]}$ values indicates a decrease in $\delta^{13}$CO$_{OM}$ at higher $\gamma$. Laws et al. (1997) reasoned that active HCO$_3^-$ uptake would introduce a positive shift in $\delta^{13}$CO$_{OM}$ and is hence inconsistent with the observed downward shift. This reasoning neglects that an increased $\gamma$ 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 $\delta^{13}$CO$_{OM}$ 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 $\delta^{13}$CO$_{OM}$ data as a function of $\nu_{[CO_2]}$ 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 $\nu_{[CO_2]}$ and $\epsilon_p$ nor an upward curvature of $\epsilon_p$ with $\nu_{[CO_2]}$ can exclude active HCO$_3^-$ or CO$_2$ uptake. Whether HCO$_3^-$ or CO$_2$ is actively taken up by a particular microalga cannot presently be decided on the basis of isotope data (except for the case when $\delta^{13}$CO$_{OM}$ is higher than
indicating that HCO$_3^-$ is a significant carbon source. Available models of carbon isotopic fractionation are insufficiently constrained to distinguish between these possibilities.

Acknowledgements. We are grateful to G. H. Rau and P. D. Tortell for helpful discussions.

LITERATURE CITED


Eppley RW (1972) Temperature and phytoplankton growth in the sea. Fish Bull 70:1063–1085


Submitted: December 23, 1998; Accepted: April 20, 1999

Proofs received from author(s): May 18, 1999