Relationship between Carbon Isotope Discrimination and the Specific Growth Rate of Green Alga *Chlamydomonas reinhardtii*

Kazushi TAKAHASHI, Eitaro WADA and Mitsuru SAKAMOTO

Abstract

The relationship between carbon isotope discrimination and the specific growth rate of a green alga *Chlamydomonas reinhardtii* Dangeard (IAM C-238) was studied in the continuous culture system under constant CO₂ pressure. In both the nitrate-limited and the light-limited conditions, carbon isotope discrimination ($\Delta \delta^{13}C$) between alga and CO₂ was dependent upon the specific growth rate ($\mu$) of alga. The algal $\delta^{13}C$ value increased with the specific growth rate ($\mu$). In natural environments, the variations in carbon isotope ratio of phytoplankton or particulate organic carbon may be mainly attributed to change in the specific growth rate of phytoplankton, at least for the case in which the $\delta^{13}C$ values for CO₂ (aq) and HCO₃⁻ in water are kept constant.

Key words: *Chlamydomonas reinhardtii*, carbon isotope ratio, specific growth rate, carbon isotope discrimination

1. Introduction

It is generally recognized that carbon isotope discrimination occurs during photosynthetic carbon fixation (PARK and EPSTEIN, 1960). In terrestrial ecosystems, the magnitude of the carbon isotope ratio ($\delta^{13}C$) in plants was primarily determined by the type of carboxylating enzyme; C₃ pathway or C₄ pathway. Environmental and biological factors also influence the carbon isotope ratio of plants (O'LEARY, 1981). O'LEARY (1981) and BERRY (1988) introduced a steady state model of carbon isotope discrimination ($\Delta \delta^{13}C$) during photosynthesis of C₃ pathway, and suggested that $\Delta \delta^{13}C$ in plants was controlled by the transport rate and carboxylating rate of inorganic carbon. The CO₂ partial pressure ($P_{CO₂}$) of feeding gas was confirmed to be a major factor affecting $\delta^{13}C$ values of algae (MIZUTANI and WADA, 1982; CALDER and PARKER, 1973; PARDOU *et al*., 1976), supporting that carbon isotope discrimination was partly governed by diffusion flux of dissolved inorganic carbon (DIC) into cells. On the other hand, DEGENS *et al*. (1968) and DEUSER *et al*. (1968) reported that under constant CO₂ feeding condition, elevated $\delta^{13}C$ values of algae resulted from increase of growth rate associated with higher temperature. If the algal specific growth rate is closely correlated with dynamics between CO₂ transport into algal cell and its assimilation, we can predict that carbon isotope discrimination would also depend on the specific growth rate.

In this study, we attempted to evaluate variations in $\delta^{13}C$ value of phytoplankton in natural aquatic environments, on the base of the quantitative relationship between $\Delta \delta^{13}C$ and the specific growth rate.

2. Materials and methods

The green alga *Chlamydomonas reinhardtii* Dangeard, strain IAM C-238 obtained from the Algal Culture Collection of the Institute of Applied Microbiology, University of Tokyo, was grown in a continuous culture system.
When a steady state is achieved, the specific growth rate ($\mu$, day$^{-1}$) of algae becomes equal to the dilution rate, a ratio of inflow volume per unit time to the volume of the culture vessel. During nitrate-limited studies, the reservoir nitrate concentration in the MBM medium (Watanabe, 1960) was set at 225 $\mu$M, as opposed to 1130 $\mu$M under light-limited condition. The culture vessel was continuously bubbled (3 liter•min$^{-1}$) with sterile air (0.03% CO$_2$) at 20°C under fluorescent light (12L/12D). Light intensity was controlled by changing the distance between culture vessel and fluorescent lamps. Maximal light intensity was 400 $\mu$E•m$^{-2}$•sec$^{-1}$ at the surface of the vessel. The cell number was monitored under microscopy using an aliquot of the outflows. After a steady state for the cell number was established, the algal sample was collected by the filtration through a Whatman GF/C filter precombusted (450°C). The concentrations of organic carbon and nitrogen were measured by a quadrupole mass spectrometer (Anelva TE-150) with a combustion furnace (Otsuki et al., 1985). To analyse carbon isotope ratio, organic carbon on the filter sample was converted to CO$_2$ by the method of Minagawa et al. (1984). To determine the carbon isotope ratio of feeding CO$_2$ in culture, CO$_2$ was collected from feeding air cryogenically using a liquid nitrogen trap.

The carbon isotope ratio was determined using a MAT-251 mass spectrometer. The carbon isotopic ratio is expressed in terms of $\delta^{13}$C as defined by the following equation:

$$\delta^{13}C(\%) = \frac{\left(\frac{^{13}C}{^{12}C}\right)_{\text{sample}} - 1}{\left(\frac{^{13}C}{^{12}C}\right)_{\text{standard}}} \times 1000$$

The standard is carbonate from the fossil skeleton of Belemnitella americana from the Pee Dee formation of South Carolina (PDB). The precision of $^{13}$C determination is within 0.2%.

Carbon isotope discrimination ($\Delta\delta^{13}$C) is generally expressed as a difference in the $\delta^{13}$C value between source and product:

$$\Delta\delta^{13}C = \frac{\delta^{13}C \text{ (source)} - \delta^{13}C \text{ (product)}}{1 + \delta^{13}C \text{ (source)}} / 1000$$

Nitrate concentration in the culture vessel was obtained according to the Technicon Industrial Method (1972).

3. Steady state model

The photosynthetic pathway of C$_3$ plant can be illustrated by a two-step carboxylation sequence (Fig. 1a). The first step ($F_1$ and $F_3$) is a reversible reaction which involves CO$_2$ transport across the cellular membrane by diffusion, followed by a carboxylation reaction by ribulose bisphosphate carboxylase (RuBPCase).

Under the steady state condition, isotope mass balance calculation results in the following equation, from Berry's equation (Eq. 3 p 86; Berry, 1988) modified by using $F_2 = F_1 - F_3$.

![Fig. 1. Schematic diagram of the CO$_2$ assimilation model of (a) C$_3$ plants, and (b) aquatic microalgae (e.g., C. reinhardtii), assuming that input of DIC into algal cell is mainly mediated by active transport system.](image-url)
\[
\Delta \delta^{13}C = b + (a - b) \times \frac{F_2}{F_1} \quad \text{(Eq. 1)}
\]

where \(a\) and \(b\) are discrimination factors associated with the processes of diffusion and carboxylation reaction, respectively. Thus, the magnitude of carbon isotope discrimination of C3 pathway is determined by the dynamics between the rate of carboxylation rate \(F_2\) and the supply rate \(F_1\) of substrates. In this model, the flux of CO2 diffusion process \(F_1\) is dependent on the CO2 concentration in air, while \(F_2\) as the speed of Calvin cycle is mainly affected by ambient light intensity, the intracellular CO2(aq) concentration, and RuBPCase activity.

However, it is a question whether this model (Fig. 1a) could be applied into transport and assimilation of dissolved inorganic carbon (DIC) in aquatic algae. The reason is that some aquatic algae have ability to transport bicarbonate \((\text{HCO}_3^-)\) into their cell under feeding air of normal CO2 content (0.03%) (e.g., LUCAS and BERRY, 1985a). When algal cells grown at high CO2 concentration (5%) were transferred to low CO2 condition (0.03%), the alga immediately has a high affinity for CO2. This phenomena resulted from the active transport of DIC across the cellular membranes and the carbonic anhydrase (CA) activity (LUCAS and BERRY, 1985b). CA catalyzes the interconversion of CO2 and \(\text{HCO}_3^-\): \(\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3\) (The dissociation of \(\text{H}_2\text{CO}_3\) to \(\text{HCO}_3^-\) and \(\text{H}^+\) is extremely rapid.). Although there is still considerable controversy with respect to the mechanisms of DIC utilization of most aquatic algae, a model of DIC assimilation was well established (Fig. 1b) in Chlamydomonas reinhardtii (e.g., SPALDING and PORTIS, 1985). BERRY (1988) showed a simple model of the CO2 concentrating system of green alga Chlamydomonas reinhardtii and proposed the following equation, assuming very small fractionation at active transport of \(\text{HCO}_3^-\) \((a = 0, \text{Eq. 1})\) can be ignored.

\[
\Delta \delta^{13}C = (c + d) + b - b \times \frac{F_2}{F_1} \quad \text{(Eq. 2)}
\]

where \(c\) and \(d\) are the discriminations occurring in the dissolution of CO2, 1.1% (O'LEARY, 1984) and in the equilibrium conversion of gaseous CO2 to \(\text{HCO}_3^-\), -9.0% (MOOK et al., 1974), respectively. For C3 carboxylation, it is thought \(b = 29.4\%\) (ROESKE and O'LEARY, 1984). Consequently, Eq. 2 can be rewritten as follows:

\[
\Delta \delta^{13}C = 21.5 - 29.4 \times \frac{F_2}{F_1} \quad \text{(Eq. 3)}
\]

4. Results and discussion

4-1. Relationship between \(\Delta \delta^{13}C\) and \(\mu\) in C. reinhardtii

The specific growth rates under the nitrate-limited and light-limited conditions ranged from 0.15 to 0.55 and from 0.13 to 0.48 (day\(^{-1}\)), respectively. Substantial concentrations of nitrate were present (>895 \(\mu\)M) in the vessels on light-limited experiments, while nitrate was not detected (<0.03 \(\mu\)M) in the nitrate-limited condition, by our conventional nutrient analyses (Table 1). Although a small number of bacteria coexisted in the algal culture, the contribution of bacterial carbon was much less because of high algal density \((10^6-10^7 \text{ cells} \cdot \text{l}^{-1})\). Physiological variables of algal growth are influenced by the growth limiting factors, for example, light intensity, dissolved inorganic nitrogen concentration, and temperature. LAWS and BANNISTER (1980) showed that C/N ratio was linearly correlated with the specific growth rate \(\mu\) under light and nutrient limitation, but with the regressions having opposite signs. In this study, algae growing at the highest specific growth rate \((0.55\text{day}^{-1})\) under nitrate–limited condition gave a low C/N ratio (Table 1) However, no clear relationships between mean C/N ratio and \(\mu\) were found in either growth condition. The mean C/N value of algae under nitrate–limited condition of 9.0 (range, 6.5–10.4) was higher than light–limited condition of 4.8 (range, 4.5–5.1). This result strongly suggested that algae in light–limited condition were not nitrogen-deficient.

Carbon isotope ratio \((\delta^{13}C)\) of CO2 in feeding air was \(-9.6\%\), while carbon isotope ratios of the algal cell varied considerably, ranging
Table 1. POC/PON ratios, the $\Delta^{13}C$ values, $\Delta^{13}C$, and $F_2/F_1$ ratio (calculated from Eq. 3) of Chlamydomonas reinhardtii, and nitrate concentrations ($\mu$M) in culture vessel, in various specific growth rates ($\mu$, day$^{-1}$).

<table>
<thead>
<tr>
<th>$\mu$</th>
<th>C/N</th>
<th>NO$_3^-$</th>
<th>$\delta^{13}C$</th>
<th>$\Delta^{13}C$</th>
<th>$F_2/F_1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.15</td>
<td>9.2</td>
<td>&lt;0.03</td>
<td>-33.4</td>
<td>24.4</td>
<td>-0.11</td>
</tr>
<tr>
<td>0.15</td>
<td>8.8</td>
<td>&lt;0.03</td>
<td>-29.3</td>
<td>20.3</td>
<td>0.03</td>
</tr>
<tr>
<td>0.25</td>
<td>10.4</td>
<td>&lt;0.03</td>
<td>-24.9</td>
<td>15.8</td>
<td>0.18</td>
</tr>
<tr>
<td>0.25</td>
<td>10.3</td>
<td>&lt;0.03</td>
<td>-24.1</td>
<td>15.0</td>
<td>0.21</td>
</tr>
<tr>
<td>0.38</td>
<td>6.5</td>
<td>&lt;0.03</td>
<td>-16.9</td>
<td>7.8</td>
<td>0.46</td>
</tr>
<tr>
<td>0.55</td>
<td>5.2</td>
<td>940</td>
<td>-27.9</td>
<td>18.9</td>
<td>0.07</td>
</tr>
<tr>
<td>0.45</td>
<td>4.6</td>
<td>895</td>
<td>-19.0</td>
<td>9.9</td>
<td>0.39</td>
</tr>
<tr>
<td>0.48</td>
<td>4.5</td>
<td>943</td>
<td>-17.6</td>
<td>8.5</td>
<td>0.43</td>
</tr>
</tbody>
</table>

from $-33.4\%$ to $-16.9\%$ (Table 1). Calculated carbon isotope discrimination ($\Delta^{13}C$) between alga and CO$_2$ was inversely correlated with the specific growth rate, irrespective of the growth limited conditions (Fig. 2). In nitrate-limited condition, discrimination decreased with the specific growth rate, ranging from 0.15 to 0.38 day$^{-1}$, but $\Delta^{13}C$ at 0.55 showed about the same value at 0.38 day$^{-1}$. On the other hand, in light-limited condition, $\Delta^{13}C$ appears to be inversely proportional to the specific growth rate.

In the presence of suitably high concentrations of nitrogen source, Chlamydomonas reinhardtii reproduce asexually by mitotic divisions of the haploid vegetative cells. In nitrogen-starved condition, vegetative cells undergo gametic differentiation (Kates and Jones, 1964). If gametes mix with the vegetative cell population, it might partly be attributed to the $\delta^{13}C$ value of algal carbon. Unfortunately, we could not confirm whether gametic differentiation occurred in our nitrate-limited condition.

However, Bender and Berge (1979) reported lower carbon isotope discriminations of Timothy (Phleum pratense L.) under the most optimum nutrient growth conditions. This finding corroborates the inverse relationship between $\Delta^{13}C$ and $\mu$ in nitrogen-limited condition, as observed in this study.

In continuous culture studies of Thalassiosira fluviatilis, it was confirmed that the cellular photosynthetic rate was directly proportional to the specific growth rate under both light- and nitrate-limited conditions, by calculating from the data set of Laws and Bannister (1980). Therefore, we can assume that the carboxylation rate ($F_2$) (analogous to cellular photosynthetic rate) was proportional to the specific growth rate, irrespective of the growth limited conditions. Moreover, we can predict that $F_2/F_1$ of Eq. 3 is proportional to $\mu$, since $F_1$ seems to be independent of $\mu$. If so, the relation between $\Delta^{13}C$ and $\mu$ from the data set in both growth conditions can be permitted as a linear form of Eq. 2, although available data do not suffice for an accurate representation of this relation (Fig. 2).

$$\Delta^{13}C = -35.4 \times \mu + 25.3 \ (r = -0.92) \ (Eq. 4)$$

From the comparison of Eq. 3 with Eq. 4, we can calculate the $F_2/F_1$ ratio in various specific growth rates (Table 1). The ratio $F_2/F_1$ means the proportion of fixed carbon in carbon taken up into an algal cell. We can thus define $F_2/F_1$ as the carbon fixation efficiency within the cell. From data in Sharkey and Berry (1985), we calculated the $F_2/F_1$ ratio to be 0.58 in C. reinhardtii at low CO$_2$ condition. Calculated $F_2/F_1$ ratios in this study by Eq. 4 ranged from $-0.11$ to 0.46 in nitrate limitation, and from 0.07 to 0.43 in light limitation (Table 1). At 0.15 (day$^{-1}$) of $\mu$, $F_2/F_1$ was less than zero, for some unknown reason. With increase in
Carbon Isotope Ratio and Specific Growth Rate of Phytoplankton

Fig. 2. Relationship between carbon isotope discrimination and the specific growth rate ($\mu$) in green alga *Chlamydomonas reinhardtii* Dangeard (IAM C-238). 
(●): Nitrate-limited condition. 
(○): Light-limited condition. 
Linear regression corresponds to: 
$$\Delta \delta^{13}C = -35.4 \times \mu + 25.3 \quad (r = -0.92).$$

the specific growth rate, the increasing percentage of DIC pool taken up into cells would presumably be fixed in organic matter. In other words, the leakiness mentioned by *FARQUHAR* (1983) would decrease with increase in the specific growth rate. *OGAWA* and *INOUE* (1983) showed that the operation of the DIC pump requires energy apparently supplied by photosystem I. *SHARKEY* and *BERRY* (1985) suggested that energy cost associated with the DIC pump operation could be offset by reduced photorespiration and increased photosynthesis. However, the observed high efficiency of carbon fixation within DIC pool might suggest that the acceleration of the DIC pump was not as high as that of the carboxylation rate at high specific growth rate.

### 4-2. Natural environments

*SACKETT et al.* (1965) and *FONTUGNE* and *DUPLESSY* (1981) found that plankton in colder Antarctic waters exhibited low $\delta^{13}C$ (about 31 %o) values and suggested a positive correlation between carbon isotope ratio of plankton and water temperature. However, *GEARING et al.* (1984) reported no direct effect of water temperature on phytoplankton $\delta^{13}C$ in the temperate estuary. The temperature effect of $\delta^{13}C$ value for plankton or particulate organic carbon (POC) was still obscure. Nevertheless, the low $\delta^{13}C$ value could surely be attributed partly to a decrease of $\mu$ in phytoplankton, due to low temperature.

*RAU et al.* (1989) proposed that $\delta^{13}C$ variations in plankton, especially latitudinal variations, are primarily attributable to ambient CO$_2$ (aq) concentration in marine environments. Indeed, in algal culture, a high P$_{CO_2}$ in medium was reported to increase $\delta^{13}C$ difference between aerated CO$_2$ and algae (*CALDER* and *PARKER*, 1973). These results corroborated the concept that $\Delta \delta^{13}C$ variation was partly governed by change in diffusion flux (F1) of CO$_2$ (aq) into algal cells, determined from P$_{CO_2}$ in medium. In natural environments, however, the CO$_2$ (aq) concentration in the euphotic zone was kept at a low level. Especially, in open ocean, CO$_2$ (aq) concentration ranged from 10 to 40 $\mu$M. Since Km value (a half saturation constant) of CO$_2$ (aq) for RuBPCase is 12 to 240 $\mu$M (*KERBY* and *RAVEN*, 1985), the rate of carboxylation reaction may depend on intracellular CO$_2$ (aq) in marine environments. Thus, even if the ambient CO$_2$ concentration increases to 40 $\mu$M, the ratio F2/F1 is not significantly depleted, because the F2 elevation is associated with increasing intracellular CO$_2$ concentration. Therefore, although $\delta^{13}C$ variation in plankton are apparently correlated with ambient CO$_2$ (aq) concentration in natural environments, the CO$_2$ (aq) variation could not directly affect the $\Delta \delta^{13}C$ in photosynthesis, except for extremely low (*TAKAMASHI et al.*, 1990a) or high DIC conditions. One must note that the P$_{CO_2}$ dependent variation in phytoplankton $\delta^{13}C$ could arise from only extraordinarily high P$_{CO_2}$ (>30 times P$_{CO_2}$ in the present atmosphere) in feeding gas (*MIzUTANI* and *WADA*, 1982), which could not exist in the euphotic zone in the natural aquatic environments.

The HCO$_3^-$ active transport was suggested to be widespread in many algal species, by supporting the C$_4$-like physiology in marine and fresh-
water algae (Kerry and Raven, 1985). With
the CO₂ concentrating system, photosynthetic
activity is generally not limited by CO₂ (aq) and
HCO₃⁻ concentrations in freshwater and
marine environments. Even if algae take
HCO₃⁻ up into cells, the δ¹³C value is mainly
affected by the specific growth rate of algae
because of the relatively constant concentration
in HCO₃⁻ in euphotic zone in lake and ocean.

In summary, the large change in the specific
growth rate of phytoplankton in natural eco-
systems would greatly contribute to plankton
δ¹³C variations. Conversely, δ¹³C variations in
phytoplankton and particulate organic matter
in natural aquatic environments can thus pro-
vide a basis for understanding the specific
growth rate. From Eq. 4, 1 % depletion in
discrimination corresponds to 0.03 (day⁻¹)
increase of the specific growth rate. The
range of δ¹³C values in marine particulate
organic matter was estimated to be from -24
to -18 % in temperate shelf and estuarine
waters (Deuser et al., 1968; Haines and
Monteague, 1979; Tan and Strain, 1983; Gearing
et al., 1984). If one uses -7 % as the δ¹³C
value of CO₂ (aq) in seawater (Craig, 1953), the
δ¹³C values correspond to μ values from 0.23 to
0.40 (day⁻¹). These ranged slightly lower than
those obtained in previous studies by other
techniques (e.g., Eppley, 1980). For example,
Durbin et al. (1975) estimated doubling rate of
0.4 to 1.94 (doublings•day⁻¹) for phytoplankton
assemblages in Narragansett Bay, corre-
sponding to the specific growth rate of 0.28 to 1.34
(day⁻¹). In lake, the δ¹³C value of phyto-
plankton has shown considerable variation, due to
the δ¹³C variation in DIC in lake water
(Takahashi et al., 1990b). In Lake Kizaki, the
seasonal change in δ¹³C value of phytoplankton
ranged from -35 to -15 % (Yoshioka et al.,
1989). But fewer δ¹³C data are available on
freshwater ecosystems. The accumulation of
more δ¹³C data may allow us to assign a
definite specific growth rate to natural phyto-
plankton assemblages.

Thus in situ measurements of primary pro-
ductivity by δ¹³C analyses may become a
powerful alternative to the conventional mea-
surements by tracer studies.

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