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Effect of Temperature on Inorganic Carbon Acquisition of *Chlamydomonas reinhardtii*

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ABSTRACT

Carbon dioxide availability for microalgae in aquatic environments increases with decreasing water temperature, while photosynthetic activity generally decreases. Therefore, inorganic carbon acquisition by algal cells is greatly affected by temperature. We investigated half-saturation constants [Km(DIC), Km(CO_2)] of inorganic carbon in photosynthesis under various temperatures for a strain of *Chlamydomonas reinhardtii*. *C. reinhardtii* showed an active carbon concentrating mechanism (CCM) at all temperature conditions investigated (5 – 25˚C), implying that CCM activity is not diminished at low temperatures. The maximum photosynthetic rate was recorded at 15˚C, while maximum CCM activity was detected at 20˚C. A higher optimum temperature for CCM activity than for photosynthesis may compensate for lower photosynthetic rates above the optimum temperature. CCM may play a more significant role at higher temperatures in algal photosynthesis in aquatic environments.

INTRODUCTION

Many microalgae, including cyanobacteria, possess a carbon-concentrating mechanism (CCM) to elevate CO_2 concentrations around the CO_2-fixing enzyme of ribulose-1, 5-bisphosphate carboxylase/oxygenase (RUBISCO), which has a relatively low affinity for CO_2 in ambient air (Kaplan and Reinhold 1999, Giordano et al. 2005). The CCM is generally activated in cells cultured under the ambient CO_2 level (0.035%) as compared to those grown under high CO_2 conditions (2-5%). Since the CCM expression requires extra energy (Beardall 1991), CCM activity possibly decreases under severe conditions, such as under low light or low temperature. Indeed, Beardall (1991) demonstrated that CCM in the cyanobacterium *Anabaena variabilis* is suppressed when cells were cultured under low amounts of light. In contrast, information on CCM activity in microalgae under low water temperature is relatively limited.

Under low temperature, CO_2 availability for microalgae tends to increase due to higher solubility. On the other hand, photosynthetic activity is generally low at lower temperatures. Therefore, the need for the active transport of DIC to maintain the supply of CO_2 to the RUBISCO active site may decrease under low temperatures. Indeed, the carbon isotope ratio, which indicates carbon fractionation in photosynthesis, is lower in polar microalgae, suggesting lower CCM activity in the polar region (Rau et al. 1989). In contrast, an increase in water temperature leads a decrease in CO_2 solubility and increase in photosynthetic activity until reaching an optimum temperature. Near the optimum temperature for photosynthesis, CCM activity may strongly affect the photosynthetic rate.

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Rapidly increasing atmospheric CO_2 levels over the last several decades probably does not boost primary productivity in aquatic environments due to the presence of CCM (Kaplan and Reinhold 1999). However, rising CO_2 levels can lead to reduced need for CCM, although CCM is still required for photosynthesis in most algal species at current CO_2 levels (0.035%). The activity is highly variable among species, and thus this physiological trait is important for determining the composition of primary producers and the primary production rate in aquatic environments. To predict the composition of primary producers in elevated CO_2 levels and temperature, CCM activity for each algal species should be considered.

The genus *Chlamydomonas* (Chlorophyceae) is one of the largest green algal genera (Pröschold et al. 2001) and has been frequently used in both ecological and plant physiological studies. The distribution extends from subtropical to polar regions. Recently we isolated a strain of *Chlamydomonas reinhardtii* from Svalvard, Norway. In the present study, we aimed to clarify the relationship between inorganic carbon acquisition and temperature using this species. The role of CCM in photosynthesis was examined by comparing the responses of CCM activity and maximum photosynthetic rate at inorganic carbon saturated conditions along a temperature gradient.

METHODS AND MATERIALS

Water was collected from a temporary arctic pond in Svalbard, Norway on 12 August 2005 (Ki et al. 2006). A portion of the sample was inoculated into C medium (Watanabe 2000) and incubated at 10°C for one week under a 12:12 hour light:dark cycle with a light intensity of approximately 50 µmol photons m^{-2} s^{-1}. A portion of the culture was spread onto agar-plated C medium (agar 1.2%) and incubated under the same conditions for three weeks. A greenish colony was transferred to a liquid C medium in a 96-well plate, and cultured under the same conditions. A unialgal culture was confirmed and was selected to establish a clonal culture, labeled as strain HYNP024. 18S rDNA sequencing revealed that the strain was a clade of *Chlamydomonas reinhardtii* (Pröschold et al. 2001); it also possessed morphological features of *C. reinhardtii*. Subsequently, we cultured HYNP024 in C medium at 10°C.

Photosynthetic O_2 evolution was measured by the Winkler method using 60 mL bottles. Generally, incubation was carried out for three hours in triplicate. Measurement of dissolved oxygen (DO) was with a DO titrator (716 DMS Titrino, Metrohm).

A portion of algal culture was filtered on GF/F filters (Whatman). The filter was soaked with 90% aceton overnight at 4°C under dark condition. Extracted chlorophyll *a* (chl *a*) was measured spectrophotometrically, and calculated according to Jeffrey and Humphery (1975).

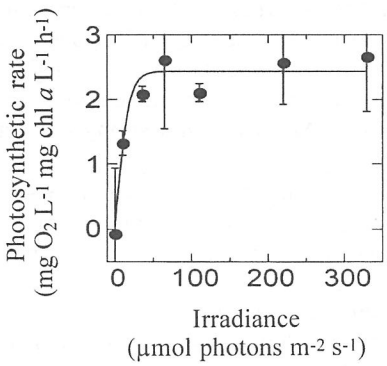

Figure 1. Photosynthesis-irradiance (P-I) curve for *C. reinhardtii* NP024. Plots are means for three replications, and vertical bars indicate standard deviations.

Before the measurement of CCM activity, we examined the photosynthesis-irradiance (P-I) curve to determine the optimum light intensity for photosynthesis. Seven steps of light intensities (0, 10, 36, 65, 110, 220, 330 μmol photons m^{-2} s^{-1}) were used. Photosynthetic rate as a function of light intensity was fitted with the Platt and Jassby model (1976).

Algal cells were cultured in a 1-L flask containing 400 mL C medium at 5, 10, 15, 20, 25°C for at least five days under the same light condition described above. Flasks were bubbled with an air pump (low- CO_2) or 5% CO_2 gas (high- CO_2). Cells were collected by centrifugation (x3000 g, 4 min.) and resuspended in the CO_2 free C medium (pH 8.0). CO_2-free medium was prepared as follows. C medium adjusted to pH 4.0, was autoclaved and bubbled with CO_2-free gas overnight and readjusted to pH 8.0 with freshly prepared NaOH solution. The absence of DIC (dissolved inorganic carbon) was confirmed with similarly prepared media using infra-red measurement.

For the measurement of photosynthetic CO_2 evolution as a function of DIC concentration, six DIC concentrations were prepared between 0 and 6.75 mM. Incubations were conducted at each temperature (5 – 25°C) under 220 μmol photons m^{-2} s^{-1} for three hours in triplicate. Parameters for inorganic carbon acquisition were calculated by fitting with the Michaelis-Menten equation and using pKa1 and pKa2 calculated from following equations: log pKa1 = -356.3094 - 0.06091964T + 21834.37 / T + 126.8339 logT - 1684915 / T^2 and log pKa2 = -107.8871 - 0.03252849T + 5151.79 / T + 38.92561 logT - 563713.9 / T^2, where T is thermodynamic temperature (K).

RESULTS AND DISCUSSION

Photosynthetic O_2 evolution was saturated above 70 μmol photons m^{-2} s^{-1} (Fig. 1). The alpha slope was 0.14 mg O_2 mg chl a^{-1} h^{-1} (μmol photons m^{-2} s^{-1})$^{-1}$. Thus, all examination of inorganic carbon acquisition was carried out at 220 μmol photons m^{-2} s^{-1}.

For the low CO_2 condition, O_2 evolution was saturated as 7.8 mg O_2 mg chl a^{-1} h^{-1} above 1.2 mM DIC at 10°C, while those for the high CO_2 condition did not saturate until 4 mM (Fig. 2). Km(DIC) for low- and high-CO_2 conditions were 0.10 and 1.31 mM, respectively (Table 1). These results clearly indicate the expression of CCM in *C. reinhardtii* HYP024. Vmax for low- and high-CO_2 conditions were 7.82 and 8.03, respectively.

Maximum photosynthetic rate (Vmax) was higher at 15 and 20°C with the highest value of 13.95 mg O_2 mg chl a^{-1} h^{-1} at 15°C (Fig. 3). Vmax suddenly dropped to 7.83 at 10°C, and the lowest value was recorded at 5°C. Both Km(DIC) and Km(CO_2) were the lowest at 20°C, indicating that CCM activity was the highest at 20°C. Both

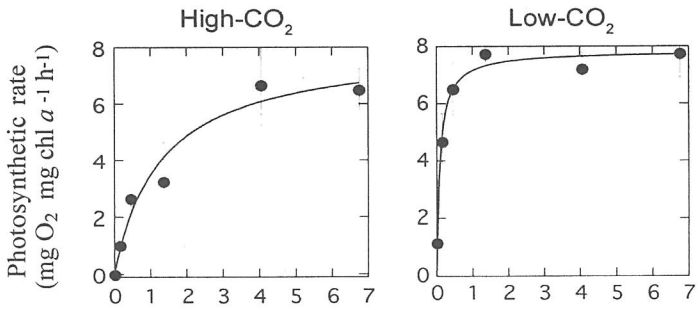

Figure 2. Photosynthetic rates under various dissolved inorganic concentrations. Curves are fitted with Michaelis-Menten equation. Plots are means for three replications, and vertical bars indicate standard deviations.

Km(DIC) and Km(CO_2) values above 15°C were less than half of those observed below 10°C. These values were higher at 20 and 25°C. Km(CO_2) for *C. reinhardtii* strain 137c⁻ at 23°C was 1.6 μM (Morita *et al.* 1998), while that for strain HYP024 was 0.6 μM at 20°C, 0.7 μM at 25°C. In addition, the Km(CO_2) values for HYP024 were within the range of values reported for other species of the *Chlamydomonas* genus (0.1 – 11.0 μM, Morita et al. 1998 and 1999).

Vmax for NP024 did not differ between low- and high-CO_2 conditions, which is consistent with previous studies (Morita et al. 1998 and 1999). Vmax values for *Chlamydomonas*/*Chloromonas* strains were not significantly different between high- and low-CO_2 conditions, although CCM expression requires extra energy (Beardall, 1991). Costs for the expression of CCM in low-CO_2 condition seemed not to appear as a decrease in Vmax. In other words, the expression of CCM does not affect the photosynthetic rate under saturated CO_2 conditions.

The Km(DIC) of cells grown in high-CO_2 condition was 1.3 mM, which implies that CCM is still required when atmospheric CO_2 concentrations increase two- or three-fold. Therefore, to predict the phytoplankton response to climate change, such as global warming and increased levels of CO_2, the affinity for inorganic carbon and the maximum photosynthetic rate for each algal species are quite important.

As previously mentioned, the need for CCM is low under low temperature due to low photosynthetic activity and high CO_2 availability. In the present study, Km(DIC) values recorded at all temperatures (0.025 – 0.119 mM) were obviously lower than those

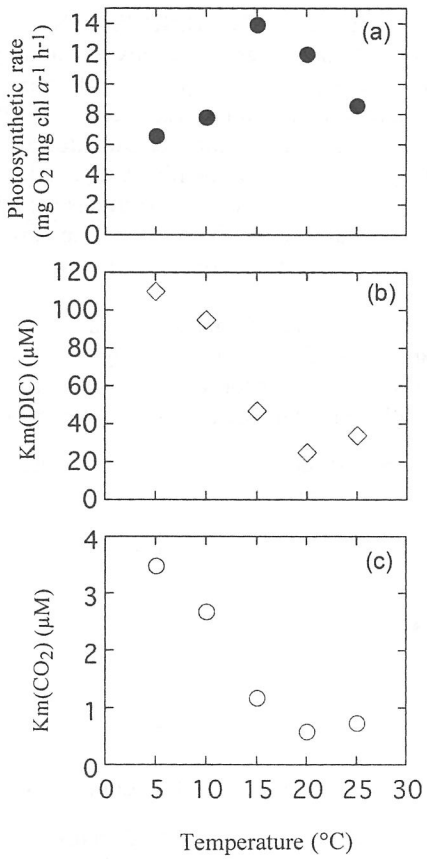

Figure 3. Temperature-dependent maximum photosynthetic rate (a), Km(DIC) (b), and Km(CO_2) (c).

under high-CO_2 conditions (1.31 mM). Therefore, it is likely that CCM of *Chlamydomonas* was activated at all temperatures investigated. Indeed, the CCM activity was not diminished in macroalgae of *Porphyra endivifolium*, and *Palmaria decipens* at 0°C (Beardall and Roberts 1999). CCM activity seems not to be diminished by low water temperature.

The highest Vmax was recorded at 15°C, while CCM activity was the highest at 20°C. In addition, when Vmax suddenly dropped to 8.61 mgO_2 mg chl a^{-1} h^{-1} at 25°C, CCM activity maintained the same level as at 20°C. These results clearly demonstrated that the optimum temperature for photosynthesis and CCM were different. At high water temperatures, CO_2 availability decreases, while the CO_2 requirement for algal cells increases due to higher levels of photosynthesis. Thus, the need for CCM is positively correlated with temperature. Higher optimum temperature for CCM activity than photosynthesis may compensate for lower photosynthetic rates above the optimum temperature.

Table 1. Half-saturation constants for inorganic carbon [Km(DIC), Km(CO_2)] and maximum photosynthetic rate (Vmax) under inorganic carbon saturation at 10°C.

	High-CO_2	Low-CO_2
Km(DIC) (μM)	100	1310
Km(CO_2) (μM)	2.82	3.69
Vmax (mg O_2 mg chl a^{-1} h^{-1})	7.83	8.05

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