CSIRO PUBLISHING
www.publish.csiro.au/journals/fpb *Functional Plant Biology*, 2004, **31**, 1027–1033

Photosynthetic utilisation of inorganic carbon and its regulation in the marine diatom *Skeletonema costatum*

Xiongwen Chen[A] *and Kunshan Gao*[B]

[A]Department of Biology, Hubei Normal University, Huangshi 435002, Hubei, China.
[B]Marine Biology Institute, Shantou University, Shantou 515063, Guangdong, China.
Corresponding author; email: ksgao@stu.edu.cn

Abstract. Photosynthetic uptake of inorganic carbon and regulation of photosynthetic CO_2 affinity were investigated in *Skeletonema costatum* (Grev.) Cleve. The pH independence of $K_{1/2}(CO_2)$ values indicated that algae grown at either ambient (12 μmol L^{-1}) or low (3 μmol L^{-1}) CO_2 predominantly took up CO_2 from the medium. The lower pH compensation point (9.12) and insensitivity of photosynthetic rate to di-isothiocyanatostilbene disulfonic acid (DIDS) indicated that the alga had poor capacity for direct HCO_3^- utilisation. Photosynthetic CO_2 affinity is regulated by the concentration of CO_2 rather than HCO_3^-, CO_3^{2-} or total dissolved inorganic carbon (DIC) in the medium. The response of photosynthetic CO_2 affinity to changes in CO_2 concentration was most sensitive within the range 3–48 μmol L^{-1} CO_2. Light was required for the induction of photosynthetic CO_2 affinity, but not for its repression, when cells were shifted between high (126 μmol L^{-1}) and ambient (12 μmol L^{-1}) CO_2. The time needed for cells grown at high CO_2 (126 μmol L^{-1}) to fully develop photosynthetic CO_2 affinity at ambient CO_2 was approximately 2 h, but acclimation to low or very low CO_2 levels (3 and 1.3 μmol L^{-1}, respectively) took more than 10 h. Cells grown at low CO_2 (3 μmol L^{-1}) required approximately 10 h for repression of all photosynthetic CO_2 affinity when transferred to ambient or high CO_2 (12 or 126 μmol L^{-1}, respectively), and more than 10 h at very high CO_2 (392 μmol L^{-1}).

Keywords: CO_2, dissolved inorganic carbon, light, photosynthesis, photosynthetic CO_2 affinity.

Introduction

In marine environments, the predominant form of dissolved inorganic carbon is HCO_3^-, and CO_2 usually accounts for less than 1% of the total DIC. The concentration of CO_2 in seawater is lower than that required for half the maximal photosynthetic rate ($K_{1/2}[CO_2]$), so diffusive entry of CO_2 would always limit photosynthesis if photosynthetic marine organisms relied entirely upon ambient dissolved CO_2 for photosynthesis. To maintain efficient photosynthesis despite the low CO_2 concentration, many phytoplankton species have developed a carbon concentrating mechanism (CCM), which increases the CO_2 concentration in the vicinity of Rubisco (Raven and Falkowski 1999).

The mechanisms of inorganic carbon uptake in cyanobacteria, green algae and diatoms have been extensively studied (Kaplan and Reinhold 1999; Badger *et al*. 2002; Colman *et al*. 2002; Shibata *et al*. 2002). In cyanobacteria, Na^+-independent and -dependent HCO_3^- uptake have been reported in *Synechococcus* species (Espie and Kandasamy 1992, 1994). In green algae, both HCO_3^- and CO_2 are taken up by *Chlamydomonas reinhardtii* Dangeard and *Chlorella* species grown at ambient CO_2 (Sültemeyer *et al*. 1991; Williams and Colman 1995; Bozzo *et al*. 2000). Diatom-dominated phytoplankton assemblages are able to take up HCO_3^- directly (Tortell *et al*. 1997, 2000) and the marine diatoms *Phaeodactylum tricornutum* Bohlin and *Thalassiosira pseudonana* Hasle & Heindal take up CO_2 and HCO_3^- (Rotatore *et al*. 1995; Burkhardt *et al*. 2001; Morel *et al*. 2002).

The regulation of the CCM in green algae and cyanobacteria is well known (Coleman 1991; Moroney and Somanchi 1999; Beardall and Giordano 2002). In the green algae *C. reinhardtii* and *Chlorella* species, growth in high CO_2 conditions (5% CO_2) repressed DIC (both HCO_3^- and CO_2) transport systems, while a transfer of cells grown at high CO_2 to ambient air induced DIC transport (Sültemeyer

Abbreviations used: AZ, acetazolamide; CA, carbonic anhydrase; CCM, carbon concentrating mechanism; DIC, dissolved inorganic carbon; DIDS, di-isothiocyanatostilbene disulfonic acid.

 10.1071/FP04076 1445-4408/04/101027

et al. 1989; Kaplan and Reinhold 1999). It has been suggested that the CO_2 concentration in the medium is a critical factor determining CCM expression (Matsuda *et al*. 1999; Bozzo and Colman 2000; Fukuzawa *et al*. 2001). In cyanobacteria, when cells grown at high CO_2 were transferred to low CO_2, they developed a CCM with a high photosynthetic affinity for external DIC, and evidence suggested that total DIC might be a key signal inducing CCM in two species of *Synechococcus* (Mayo *et al*. 1986; Sültemeyer *et al*. 1998). External CO_2 concentration may be the sensor inducing CCM in the diatom *P. tricornutum* (Johson and Raven 1996; Matsuda *et al*. 2001). Extracellular carbonic anhydrase (CA), a component of CCM in some algal species, was regulated by CO_2 concentration in the medium in the diatoms *S. costatum* (Nimer *et al*. 1998) and *T. weissflogii* (Grunow) Fryxell & Hasle (Lane and Morel 2000; Burkhardt *et al*. 2001). The study presented here examines which types of DIC the marine diatom *S. costatum* takes up, and investigates the regulation of the CCM.

Materials and methods

Skeletonema costatum (Grev.) Cleve (strain 2042) from the Institute of Oceanography, Chinese Academy of Sciences, Qingdao city, China, was cultured in filtered seawater enriched with f / 2 medium under continuous illumination of 180 μmol m^{-2} s^{-1} at 20°C. The DIC concentrations of the medium were controlled by adding $NaHCO_3$ solution into CO_2-free seawater. Changes in the DIC levels in cultures were kept within 10% by maintaining cell densities in a range of approximately $1–4\times10^4$ cells mL^{-1}, and renewing the medium every 24 h. The cells for experiments were collected during the mid-exponential growth phase.

Photosynthetic oxygen evolution was measured with a Clark-type oxygen electrode (YSI 5300, YSI Inc., Yellow Springs, OH) at 20°C and 400 μmol m^{-2} s^{-1}. Cells were harvested, washed and re-suspended ($1.5–3\times10^6$ cells mL^{-1}) in CO_2-free seawater buffered with 25 mmol L^{-1} Tris–HCl at pH 8.2 or 9.0. The CO_2-free seawater was adjusted according to Gao *et al*. (1993). Following the addition of $NaHCO_3$, the rates of oxygen evolution were measured at defined DIC concentrations. The photosynthetic $K_{1/2}$ values of DIC or CO_2 (the DIC or CO_2 concentration required to give half-maximal photosynthetic rate) were determined by fitting rates of photosynthetic oxygen evolution at various DIC or CO_2 concentrations to the Michaelis–Menten formula. The CO_2 concentrations at defined DIC levels were calculated according to Chen (1999).

For the closed-system pH drift experiment, the cells were washed and resuspended (2.8×10^5 cells mL^{-1}) in a closed bottle (80 mL) in unbuffered fresh medium of filtered natural seawater (2.0 mmol L^{-1} DIC). The pH drift of the suspension was monitored at 20°C and 180 μmol m^{-2} s^{-1}. Acetazolamide (AZ, Sigma-Aldrich China Inc., Shanghai, China) was used at a final concentration of 200 μmol L^{-1}. The pH compensation points were determined as the pH value when it no longer increased.

The effect of di-isothiocyanatostilbene disulfonic acid (DIDS) on photosynthetic oxygen evolution was measured at pH 8.2 and 2.0 mmol L^{-1} DIC at 20°C and 400 μmol m^{-2} s^{-1}. The DIDS (Sigma-Aldrich) was used at a final concentration of 200 μmol L^{-1}.

Chlorophyll *a* concentration was determined by the spectrophotometric method described by Jeffrey and Humphrey (1975).

Results

Uptake of external inorganic carbon

Net photosynthesis of *S. costatum* increased with increasing levels of DIC (or CO_2) when measured either at pH 8.2 or pH 9.0. When measured at pH 8.2, cells grown at pH 8.2 reached saturation at 2.1 mmol L^{-1} DIC (or 12.5 μmol L^{-1} CO_2) and cells grown at pH 8.7 reached saturation at 0.75 mmol L^{-1} DIC (or 4.5 μmol L^{-1} CO_2) (Fig. 1*a*). When measured at pH 9.0, net photosynthesis was saturated at higher DIC levels. Cells grown at pH 8.2 reached saturation at 13 mmol L^{-1} DIC (or 8.5 μmol L^{-1} CO_2) and cells grown at pH 8.7 cells grown at pH 9.0 reached saturation at 4.3 mmol L^{-1} DIC (or 2.8 μmol L^{-1} CO_2) (Fig. 1*b*). These findings suggest that CO_2 concentrations in the marine environment are sufficient for optimal photosynthesis if cells have been well acclimated.

For cells grown at pH 8.2, the $K_{1/2}$(DIC) value derived from the relationship of photosynthetic rate with inorganic carbon concentration measured at pH 9.0 was about 10 times greater than for that measured at pH 8.2. However, the $K_{1/2}(CO_2)$ values measured at pH 8.2 and pH 9.0 were not significantly different (Table 1). Similarly, for cells grown at pH 8.7, the $K_{1/2}$(DIC) value measured at pH 9.0 was 12.6 times greater than that measured at pH 8.2, but the $K_{1/2}(CO_2)$ was 34% higher at pH 9.0 than at pH 8.2 (Table 1). The pH independence of the $K_{1/2}(CO_2)$ values (or pH dependence of $K_{1/2}$(DIC) values) indicated that CO_2 was the main species in cellular DIC uptake. It also suggested that CO_2 was predominantly taken up from the medium for cells grown at both pH levels.

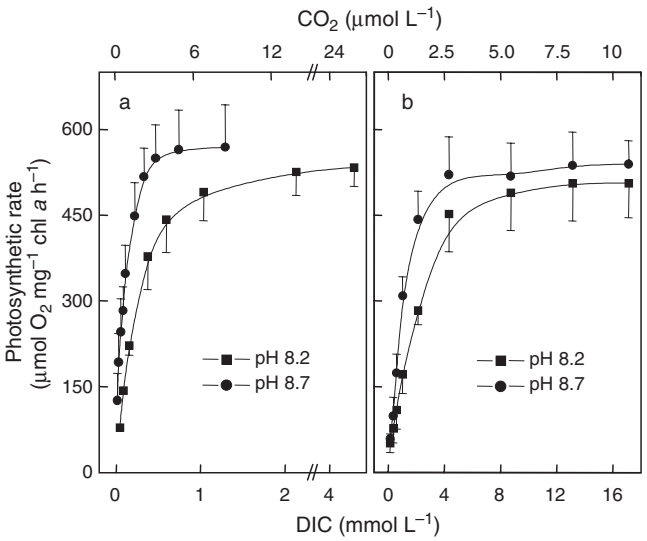

Fig. 1. Photosynthetic O_2 evolution as a function of DIC or CO_2 concentration for *S. costatum* cells grown at pH 8.2 or 8.7 and 2.0 mmol L^{-1} total DIC. Photosynthetic rates were measured at (*a*) pH 8.2 or (*b*) 9.0 at 400 μmol m^{-2} s^{-1}. Values are means ± SE ($n = 4$).

Table 1. $K_{1/2}$ values of CO_2 and DIC for *S. costatum* grown at pH 8.2 or 8.7 and 2.0 mmol L^{-1} total DIC

Photosynthetic rates were measured in the buffered medium at pH 8.2 or 9.0, at 400 μmol m^{-2} s^{-1}. Parameters were estimated from Fig. 1. Values are means ± SE ($n = 4$)

	$K_{1/2}$ value	
	DIC	CO_2
Growth pH 8.2		
Measurement pH 8.2	233 ± 13	1.39 ± 0.08
9.0	2278 ± 251	1.44 ± 0.16
Growth pH 8.7		
Measurement pH 8.2	78 ± 11	980 ± 77
0.9	0.46 ± 0.07	0.62 ± 0.05

pH compensation point and DIDS inhibition

The pH values of seawater in a closed system containing cells of *S. costatum* increased with increasing incubation time. The maximum values of 9.00 and 9.12 were obtained with and without AZ, respectively (Fig. 2).

The photosynthetic rates were 428 ± 45 and 465 ± 36 μmol O_2 mg^{-1}chlorophyll *a* h^{-1}($n = 3$) with and without DIDS, respectively, indicating that photosynthetic rates were unaffected by DIDS.

The signal regulating photosynthetic CO_2 affinity

The $K_{1/2}$ values and calculated concentrations of each DIC species in the growth media are summarised in Table 2. The $K_{1/2}(CO_2)$ values for cells grown in defined DIC concentrations at two pH levels did not show any correlation with either the pH of the media or with the DIC, HCO_3^- or CO_3^{2-} concentrations in the culture media (Table 2). By contrast, $K_{1/2}(CO_2)$ values were correlated with CO_2 concentrations in the culture media, which suggested that CO_2 in the media was the key signal for the regulation of photosynthetic CO_2 affinity.

Response of photosynthetic CO_2 affinity to changes in CO_2 concentration

The concentration of CO_2 in the media was manipulated by changing the pH and DIC concentrations (Table 3).

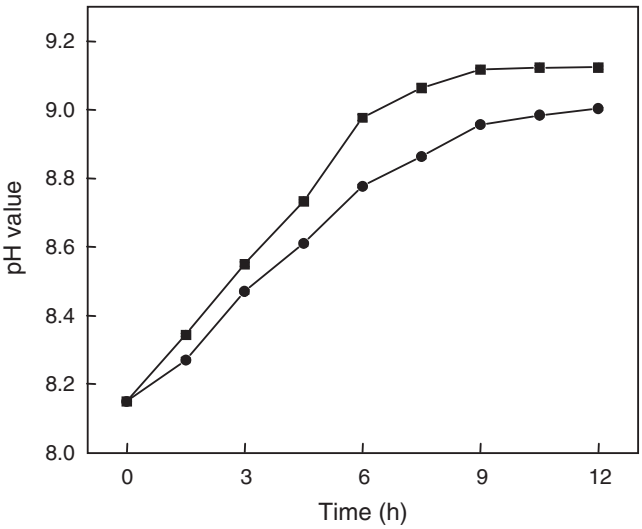

Fig. 2. Time courses of changes in pH values of seawater in a closed system caused by photosynthetic inorganic carbon uptake for *S. costatum* and the effect of adding AZ (200 μmol L^{-1}). (■) –AZ; (●) +AZ. Values are means ± SE ($n = 3$).

$K_{1/2}(CO_2)$ values increased as CO_2 concentration increased, and were most sensitive to changes in CO_2 concentration within a range 3–48 μmol L^{-1}. The CO_2 concentrations in seawater are roughly equal to atmospheric CO_2 concentrations, i.e. from 100 to 1500 μL L^{-1}, when air–water equilibrium is reached. This suggests that the sensitivity of photosynthetic CO_2 affinity to changes in CO_2 might result from long-term acclimation to changes in CO_2 concentration in seawater associated with fluctuations in atmospheric CO_2.

Regulation of photosynthetic CO_2 affinity by CO_2 and light

Steady-state photosynthesis was measured at the same CO_2 concentrations and corresponding CO_2 affinities as shown in Table 3. After 24 h of acclimation to low or high CO_2, $K_{1/2}(CO_2)$ values reached a steady state (Figs 3, 4), indicating that the time required for full induction and

Table 2. $K_{1/2}$ values of *S. costatum* cells grown under various defined DIC concentrations

Photosynthetic rates were measured at pH 8.2 at 400 μmol m^{-2} s^{-1}. Values are means ± SE ($n = 4$)

	Growth conditions (μmol L^{-1})				$K_{1/2}$ values	
pH	[DIC]	[HCO_3^-]	[CO_3^{2-}]	[CO_2]	DIC	CO_2
8.2	8000	7198	754	48	547 ± 60	3.25 ± 0.36
	2000	1800	188	12	233 ± 13	1.39 ± 0.08
	500	450	47	3	88 ± 8	0.52 ± 0.05
8.7	8000	6001	1987	12	244 ± 66	1.45 ± 0.39
	2000	1500	497	3	78 ± 11	0.46 ± 0.07

Table 3. Calculated CO_2 concentrations and $K_{1/2}(CO_2)$ values of *S. costatum* at a range of pH levels and DIC concentrations ($n = 3$ or 4)

pH	[DIC] (mmol L^{-1})	[CO_2] (μmol L^{-1})	$K_{1/2}(CO_2)$ (μmol L^{-1})
9.0	2.0	1.3	0.42 ± 0.04
8.7	2.0	3	0.46 ± 0.07
8.2	2.0	12	1.39 ± 0.08
8.2	8.0	48	3.25 ± 0.36
7.8	8.0	126	3.74 ± 0.22
7.3	8.0	392	4.31 ± 0.36

repression of the photosynthetic CO_2 affinity in *S. costatum* was less than 24 h.

When cells of *S. costatum* grown on high CO_2 media (126 μmol L^{-1}) were shifted to low CO_2 media (3 μmol L^{-1}) in the light, the $K_{1/2}(CO_2)$ values initially decreased steadily for 8 h and then reached a steady-state level of 0.44 μmol L^{-1} CO_2 after 24 h (Fig. 3*a*). There was approximately a 7-fold increase in photosynthetic CO_2 affinity after 24 h of acclimation. However, in the dark, $K_{1/2}(CO_2)$ values decreased slowly during the initial 6 h, and then increased to the same level as at $t = 0$ after 24 h of acclimation (Fig. 3*a*). It appeared that the induction of photosynthetic CO_2 affinity occurred in the dark but was severely limited, and light was required for complete induction of photosynthetic CO_2 affinity during acclimation of cells grown in high CO_2 to low

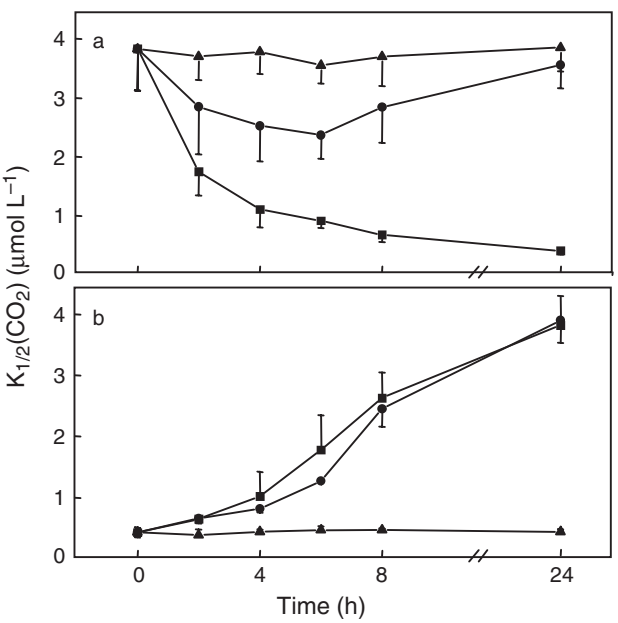

Fig. 3. Time course of changes in $K_{1/2}(CO_2)$ values of *S. costatum* cells following a transfer (*a*) from 126 to 3 μmol L^{-1} CO_2 or (*b*) vice versa in the light (180 μmol m^{-2} s^{-1}) (■), in the dark (●) and as a control, where cells were kept in 126 or 3 μmol L^{-1} CO_2 at 180 μmol m^{-2} s^{-1} (▲). Values were means ± SE ($n = 3$ or 4).

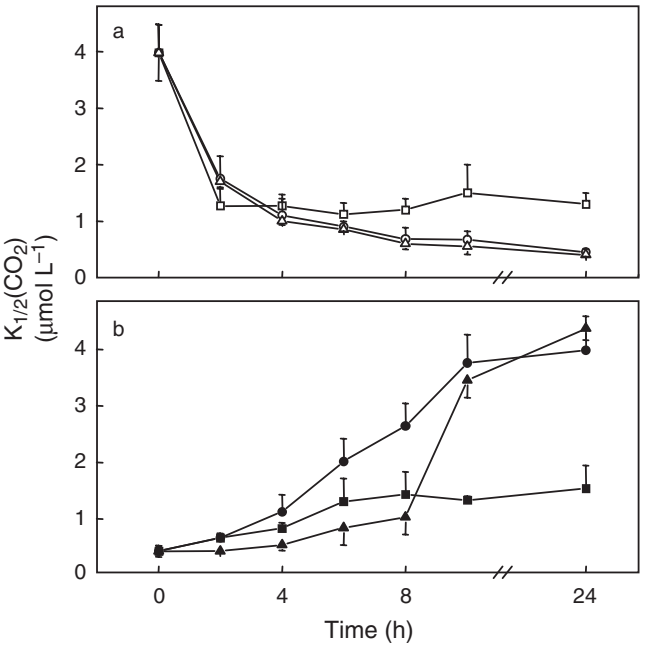

Fig. 4. Time course of changes in $K_{1/2}(CO_2)$ values of *S. costatum* cells following a shift (*a*) from 126 μmol L^{-1} CO_2 to (□) 12 μmol L^{-1} CO_2, (○) 3 μmol L^{-1} CO_2 and (△) 1.3 μmol L^{-1} CO_2 or (*b*) from 3 μmol L^{-1} CO_2 to (■) 12 μmol L^{-1} CO_2, (●) 126 μmol L^{-1} CO_2 and (▲) 392 μmol L^{-1} CO_2 in the light (180 μmol m^{-2} s^{-1}). Values were means ± SE ($n = 3$ or 4).

CO_2. By contrast, when cells grown at low CO_2 (3 μmol L^{-1}) were shifted to high CO_2 (126 μmol L^{-1}), the $K_{1/2}(CO_2)$ values showed similar behaviour in the light and in the dark (Fig. 3*b*), indicating that the repression of photosynthetic CO_2 affinity during the acclimation of cells grown at low CO_2 to high CO_2 was independent of light.

The time courses of the changes of $K_{1/2}(CO_2)$ values for cells moved in the light from high or low CO_2 media to a range of CO_2 concentrations are shown in Fig. 4. When cells grown at high CO_2 (126 μmol L^{-1}) were acclimated to ambient (12 μmol L^{-1}) or low CO_2 concentrations (3 or 1.3 μmol L^{-1}) in the light (Fig. 4*a*), the $K_{1/2}(CO_2)$ values rapidly decreased to 1.2–1.7 μmol L^{-1} CO_2 in the initial 2 h. This is equivalent to a decrease of 56–70%. The $K_{1/2}(CO_2)$ values of those cells acclimating to ambient CO_2 remained almost constant at this level during the next 22 h. In contrast, the $K_{1/2}(CO_2)$ values of the cells acclimating to the two low CO_2 concentrations continued to decrease over the next 8 h, and reached their minimum values after 24 h. The $K_{1/2}(CO_2)$ values for the two low levels of CO_2 remained very similar (Fig. 4*a*). However, the acclimation trends differed when cells were transferred from low CO_2 (3 μmol L^{-1}) to ambient (12 μmol L^{-1}) or high concentrations of CO_2 (126 and 392 μmol L^{-1}). The $K_{1/2}(CO_2)$ values during acclimation to ambient CO_2 gradually increased to about 1.3 μmol L^{-1} CO_2 over 8 h, and then stabilised (Fig. 4*b*). During acclimation to

a high CO_2 concentration (126 μmol L^{-1}), $K_{1/2}(CO_2)$ values steadily increased to about 3.70 μmol L^{-1} CO_2 after 10 h and there was no significant subsequent increase between 10 and 24 h of acclimation (Fig. 4*b*). During acclimation to the highest CO_2 concentration (392 μmol L^{-1}), the $K_{1/2}(CO_2)$ values remained almost constant for the first 2 h, then increased slowly to about 1.0 μmol L^{-1} CO_2 over the next 6 h, followed by a 2.5-fold increase in the subsequent 2 h and a slow increase from 10 h to the maximum value reached after 24 h (Fig. 4*b*).

Discussion

The DIC species within the growth medium that are used by various phytoplankton have been extensively studied. Most organisms can use CO_2 or HCO_3^- or both, but there are species-specific preferences (Kaplan and Reinhold 1999). Air-grown cells of *C. reinhardtii* preferentially take up CO_2 from the medium and HCO_3^- uptake is fully repressed at 100 μmol L^{-1} CO_2 in the medium (Sültemeyer *et al*. 1989; Bozzo and Colman 2000). Rotatore *et al*. (1995) reported that CO_2 uptake in air-grown cells of *P. tricornutum* accounted for 50% of the total DIC uptake, but Matsuda *et al*. (2001) found that air-grown *P. tricornutum* mostly used HCO_3^-. Korb *et al*. (1997) showed that air-grown cells of *S. costatum* used both HCO_3^- and CO_2 for photosynthesis. Our results showed that *S. costatum* cells grown at both low and ambient CO_2 (3 and 12 μmol L^{-1}, respectively) largely took up CO_2 from the medium. Nimer *et al*. (1998) found that extracellular CA of *S. costatum* developed only when the CO_2 concentration in the medium was less than 5.0 μmol L^{-1}, and suggested that the induction of extracellular CA occurred when the alga was cultured at 3 μmol L^{-1} CO_2. As expected, CA activity was detected in the present study (Chen and Gao 2003), but regardless of the presence of extracellular CA in *S. costatum*, CO_2 was the predominant species of DIC taken up from the medium.

Active uptake of CO_2 has been widely found among marine and freshwater microalgae and cyanobacteria (Rotatore *et al*. 1995; Kaplan and Reinhold 1999). The involvement of a CO_2 transporter has been demonstrated in cyanobacteria (Miller *et al*. 1998), green algae (Rotatore and Colman 1991) and marine diatoms (Rotatore *et al*. 1995; Burkhardt *et al*. 2001). The $K_{1/2}(CO_2)$ values measured in this study were far lower than the $K_{1/2}(CO_2)$ values for Rubisco in marine diatoms (30–60 μmol L^{-1}) reported by Badger *et al*. (1998), suggesting that *S. costatum* has the capacity to actively take up CO_2 from the medium.

A pH compensation point over 9.2 (equivalent to 0.6 μmol L^{-1} CO_2 in seawater) has been considered an indicator of HCO_3^- use in macroalgae, and the higher the pH compensation point, the greater the ability to use HCO_3^- (Axelsson and Uusitalo 1988). Several red macroalgae dependent on CO_2 diffusion for their photosynthesis were found to have final pH values less than 9.2 (Johnston *et al*. 1992). The marine diatom *P. tricornutum*, which is capable of directly using HCO_3^- (Rotatore *et al*. 1995; Burkhardt *et al*. 2001; Matsuda *et al*. 2001), had a pH compensation point of 10.3 (data not shown). In our experiment, *S. costatum* had a pH compensation point of 9.12, suggesting that its ability to directly use HCO_3^- would be poor. The pH compensation point decreased slightly in the presence of AZ, indicating that extracellular CA had little effect on the pH compensation point. Photosynthetic rates were not affected by DIDS, an inhibitor of anion exchange type mechanisms that block direct HCO_3^- uptake in marine algae (Merrett *et al*. 1996; Young *et al*. 2001), providing further evidence that *S. costatum* has little ability to directly use HCO_3^-.

The critical factor determining CCM expression in *Synechococcus* species is the HCO_3^- concentration in the bulk medium (Mayo *et al*. 1986; Sültemeyer *et al*. 1998), but in *Chlorella* spp., and *C. reinhardtii*, the signal for regulation of the CCM is a critical CO_2 concentration in the bulk medium (Matsuda and Colman 1995; Matsuda *et al*. 1998; Bozzo and Colman 2000; Bozzo *et al*. 2000; Fukuzawa *et al*. 2001). Matsuda *et al*. (2001, 2002) demonstrated that the CCM in the marine diatom *P. tricornutum* is triggered by the CO_2 concentration of the medium. Overall, these results suggest that the CCM of aquatic photoautotrophs might be regulated by a CO_2- or HCO_3^--sensing mechanism. Our results showed that photosynthetic CO_2 affinity was correlated with the CO_2 concentration of the media during acclimation, but did not vary with changes in HCO_3^-, CO_3^{2-} or total DIC concentrations, indicating that the CO_2 concentration in the medium regulates photosynthetic CO_2 affinity in the marine diatom *S. costatum*. Matsuda *et al*. (2001) suggested that the type of the inorganic carbon signal used by an organism may be an adaptation to a particular habitat. In the case of neritic species such as *P. tricornutum*, which grow in an environment with marked changes in CO_2 concentration but relatively constant bicarbonate concentration, it may be advantageous to respond to changes in CO_2 concentration. Likewise, a CO_2-sensing mechanism may be advantageous to the survival of the coastal marine diatom *S. costatum*.

There are contradictory reports on whether green algae require light to induce the CCM. During adaptation to air, light was found to be essential for the induction of photosynthetic CO_2 affinity in *Chlorella regularis* and the induction of bicarbonate transport in *C. reinhardtii* (Spalding and Ogren 1982; Umino *et al*. 1991). However, during acclimation to low CO_2 in darkness, active DIC transporters were induced in *C. reinhardtii* and *Chlorella* spp., although the rate of induction appeared to be slower than that in light (Matsuda and Colman 1995; Matsuda *et al*. 1998; Bozzo and Colman 2000), indicating that light was not an absolute requirement for induction of the CCM in the two species. There is little information on whether or not light is required

for the induction of CCM in marine diatoms. Our results showed that in darkness, photosynthetic CO_2 affinity was induced within 6 h by low CO_2, but the degree of induction was far lower than that in light. However, after 6 h at low CO_2, the photosynthetic CO_2 affinity began to decrease and after 24 h reached the same level as before transfer, which indicated that light was required for the full induction of photosynthetic CO_2 affinity in *S. costatum* (Fig. 3*a*).

Information about the repression of the CCM of microalgae in light is also scarce. Our results showed that during the acclimation from low CO_2 to high CO_2, the repression of photosynthetic CO_2 affinity was independent of light, which suggested that photosynthesis and its metabolic products did not contribute to the repression of the photosynthetic CO_2 affinity in *S. costatum*.

The time required to fully express DIC transport depends on the species. The marine diatom *P. tricornutum* took approximately 24 h to fully develop DIC transport during acclimation of cells grown at 5% CO_2 to air (Matsuda *et al*. 2001). In contrast, full induction of DIC transport in the green algae *Chorella ellispoidea* and *C. reinhardtii* was about 3–6 h (Sültemeyer *et al*. 1991; Matsuda and Colman 1995). Our results showed that when cells of *S. costatum* grown in high CO_2 were shifted to ambient CO_2, photosynthetic CO_2 affinity was fully induced within the first 2 h, but during acclimation to low CO_2 levels, photosynthetic CO_2 affinity reached 80% of the maximum within 10 h and reached the maximum after 24 h. Thus, the time required for the full induction of photosynthetic CO_2 affinity in *S. costatum* varied with the degree of acclimation.

Time is required to fully repress CCM in green algae when cells grown at low CO_2 are transferred to high CO_2. It took about 40 h for extracellular CA activity in *C. reinhardtii* to decline sufficiently when air-grown cells were shifted to high CO_2 (Fett and Coleman 1994), and in *Chlorella ellipsoidea*, it took about 24 h for DIC transport to be repressed when air-grown cells were transferred to high CO_2. (Matsuda and Colman 1995). There are few reports on the time required to repress CCM in marine microalgae. Our results showed that at least 10 h was required to fully repress photosynthetic CO_2 affinity when cells of *S. costatum* grown at low CO_2 were acclimated to ambient or high CO_2 concentrations. Matsuda and Colman (1995) suggested that the repression of DIC transport when air-grown cells of *C. ellipsoidea* were transferred to high CO_2 resulted from a high external CO_2 concentration, and the long time period required may be a function of the half-life of the transport protein in this alga. It is unclear whether a similar mechanism exists in the marine diatom *S. costatum*.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant No. 39830060).

References

Axelsson L, Uusitalo J (1988) Carbon acquisition strategies for marine macroalgae. Part I: utilization of proton exchanges visualized during photosynthesis in a close system. *Marine Biology* **97**, 295–300.

Badger MR, Andrews TJ, Whitney SM, Ludwig M, Yellowlees DC, Leggat W, Price GD (1998) The diversity and coevolution of Rubisco, plastid, pyrenoids and choroplast-based CO_2-concentrating mechanisms in algae. *Canadian Journal of Botany* **76**, 1052–1071. doi: 10.1139/CJB-76-6-1052

Badger MR, Hanson D, Price GD (2002) Evolution and diversity of CO_2 concentrating mechanisms in cyanobacteria. *Functional Plant Biology* **29**, 161–173. doi: 10.1071/PP01213

Beardall J, Giordano M (2002) Ecological implications of microalgal and cyanobacterial CO_2 concentrating mechanisms, and their regulation. *Functional Plant Biology* **29**, 335–347. doi: 10.1071/PP01195

Bozzo GG, Colman B (2000) The induction of inorganic carbon transport and external carbonic anhydrase in *Chlamydomonas reinhardtii* is regulated by external CO_2 concentration. *Plant, Cell and Environment* **23**, 1137–1144. doi: 10.1046/J.1365-3040.2000.00623.X

Bozzo GG, Colman B, Matsuda Y (2000) Active transport of CO_2 and bicarbonate is induced in response to CO_2 concentration in the green alga *Chlorella kessleri. Journal of Experimental Botany* **51**, 1341–1348. doi: 10.1093/JEXBOT/51.349.1341

Burkhardt S, Amoroso G, Riebesell U, Sültemeyer DF (2001) CO_2 and HCO_3^- uptake in marine diatoms acclimation to different CO_2 concentrations. *Limnology and Oceanography* **46**, 1378–1391.

Chen X, Gao K (2003) Effect of CO_2 concentrations on the activity of photosynthetic CO_2 fixation and extracellular carbonic anhydrase in the marine diatom *Skeletonema costatum. Chinese Science Bulletin* **48**, 2616–2620. doi: 10.1360/03WC0084

Chen ZD (1999) Inorganic carbon in seawater. In 'Principles and application of marine chemistry — marine chemistry in the coastal water of China'. (Eds ZB Zhang, ZD Chen, LS Liu and ZD Wang) pp. 106–111. (Marine Press: Beijing)

Coleman JR (1991) The molecular and biochemical analyses of CO_2-concentrating mechanisms in cyanobacteria and microalgae. *Plant, Cell and Environment* **14**, 861–867.

Colman B, Huertas IE, Bhatti S, Dason JS (2002) The diversity of inorganic carbon acquisition mechanisms in eukaryotic microalgae. *Functional Plant Biology* **29**, 261–270. doi: 10.1071/PP01184

Espie GS, Kandasamy RA (1992) Na^+-independent HCO_3^- transport and accumulation in the cyanobacterium *Synechococcus* UTEX 625. *Plant Physiology* **98**, 560–568.

Espie GS, Kandasamy RA (1994) Monensin inhibition of Na^+-dependent HCO_3^- transport distinguishes it from Na^+-independent HCO_3^- transport and provides evidence for Na^+/HCO_3^- symport in the cyanobacterium *Synechococcus* UTEX 625. *Plant Physiology* **104**, 1419–1428.

Fett JP, Coleman JR (1994) Regulation of periplasmic carbonic anhydrase expression in *Chlamydomonas reinhardtii* by acetate and pH. *Plant Physiology* **106**, 103–108.

Fukuzawa H, Miura K, Ishizaki K, Kucho K-I, Saito T, Kohinata T, Ohyama K (2001) Ccm1, a regulatory gene controlling the induction of a carbon concentrating mechanism in *Chlamydomonas reinhardtii* by sensing CO_2 availability. *Proceedings of the National Academy of Sciences USA* **98**, 5347–5352. doi: 10.1073/PNAS.081593498

Gao K, Aruga Y, Asada K, Ishihara T, Akano T, Kiyohara M (1993) Calcification in the articulated coralline alga *Corallina pilulifera*, with special reference to the effect of elevated CO_2 concentration. *Marine Biology* **117**, 129–132.

Jeffrey SW, Humphrey GF (1975) New spectrophotometric equations for determining chorophylls *a*, *b*, c_1, and c_2 in higher plants, algae and natural phytoplankton. *Biochemie und Physiologie der Pflanzen* **167**, 191–194.

Johnston AM, Marberly SC, Raven JA (1992) The acquisition of inorganic carbon by four red macroalgae. *Oecologia* **92**, 317–326.

Johson AM, Raven JA (1996) Inorganic carbon accumulation by the marine diatom *Phaeodactylum tricornutum. Europeanal Journal of Phycology* **31**, 285–290.

Kaplan A, Reinhold L (1999) CO_2 concentrating mechanisms in photosynthetic microorganisms. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**, 539–570. doi: 10.1146/ANNUREV.ARPLANT.50.1.539

Korb RE, Saville PJ, Johnston AM, Raven JA (1997) Sources of inorganic carbon for photosynthesis by three species of marine diatom. *Journal of Phycology* **33**, 433–440.

Lane TW, Morel FMM (2000) Regulation of carbonic anhydrase by zinc, cobalt, and carbon dioxide in the marine diatom *Thalassiosira weissflogii. Plant Physiology* **123**, 345–352. doi: 10.1104/PP.123.1.345

Matsuda Y, Colman B (1995) Induction of CO_2 and bicarbonate transport in the green algae *Chlorella ellipsoidea*. II. Evidence for induction in response to external CO_2 concentration. *Plant Physiology* **108**, 253–260.

Matsuda Y, Bozzo GG, Colman B (1998) Regulation of dissolved inorganic carbon transport in green algae. *Canadian Journal of Botany* **76**, 1072–1083. doi: 10.1139/CJB-76-6-1072

Matsuda Y, Williams TG, Colman B (1999) Quantification of the rate of CO_2 formation in the periplasmic space of microalgae during photosynthesis. *Plant, Cell and Environment* **22**, 397–406. doi: 10.1046/J.1365-3040.1999.00399.X

Matsuda Y, Hara T, Colman B (2001) Regulation of the induction of bicarbonate uptake by dissolved CO_2 in the marine diatom *Phaeodactylim tricornutum. Plant, Cell and Environment* **24**, 611–620. doi: 10.1046/J.1365-3040.2001.00702.X

Matsuda Y, Satoh K, Harada H, Satoh D, Hiraoka Y, Hara T (2002) Regulation of the expressions of HCO_3^- uptake and intracellular carbonic anhydrase in response to CO_2 concentration in the marine diatom *Phaeodactylum sp. Functional Plant Biology* **29**, 279–287. doi: 10.1071/PP01186

Mayo WP, Williams TG, Birch DG, Turpin DH (1986) Photosynthetic adaptation by *Synechococcus leopoliensis* in response to exogenous dissolved inorganic carbon. *Plant Physiology* **80**, 1038–1040.

Merrett MJ, Nimer NA, Dong LF (1996) The utilization of bicarbonate ions by the marine microalgae *Nannochloropsis oculata* (Droop) Hibberd. *Plant, Cell and Environment* **19**, 478–484.

Miller AG, Espie GS, Canvin DT (1998) Active transport of CO_2 by the cyanobacterium *Synechococcus* UTEX625. *Plant Physiology* **86**, 677–683.

Morel FMM, Cox EH, Kraepiel AML, Lane TW, Milligan AJ, Reinfelder JR, Tortell PD (2002) Acquisition of inorganic carbon by the marine diatom *Thalassiosira weissflogii. Functional Plant Biology* **29**, 301–308. doi: 10.1071/PP01199

Moroney JV, Somanchi A (1999) How do algae concentrate CO_2 to increase the efficiency of photosynthetic carbon fixation? *Plant Physiology* **119**, 9–16. doi: 10.1104/PP.119.1.9

Nimer NA, Warren M, Merrett MJ (1998) The regulation of photosynthetic rate and activation of extracellular carbonic anhydrase under CO_2-limiting concentrations in the marine diatom *Skeletonema costatum. Plant, Cell and Environment* **21**, 805–812. doi: 10.1046/J.1365-3040.1998.00321.X

Raven JA, Falkowski PG (1999) Oceanic sinks for atmospheric CO_2. *Plant, Cell and Environment* **22**, 741–755. doi: 10.1046/J.1365-3040.1999.00419.X

Rotatore C, Colman B (1991) The active uptake of carbon dioxide by the unicellular green algae *Chlorella saccarophila* and *C. ellipsoidea. Plant, Cell and Environment* **14**, 371–375.

Rotatore C, Colman B, Kuzma M (1995) The active uptake of carbon dioxide by the marine diatoms *Phaeodactylum tricornutum* and *Cyclotella* sp. *Plant, Cell and Environment* **18**, 913–918.

Shibata M, Ohkawa H, Katoh H, Shimoyama M, Ogawa T (2002) Two CO_2 uptake systems in cyanobacteria: four systems for inorganic carbon acquisition in *Synechocystis* sp. Strain PCC 6803. *Functional Plant Biology* **29**, 123–129. doi: 10.1071/PP01188

Spalding MH, Ogren W (1982) Photosynthesis is required for induction of the CO_2-concentrating system in *Chlamydomonas reinhardtii. FEBS Letters* **145**, 41–44. doi: 10.1016/0014-5793(82)81202-7

Sültemeyer DF, Millar AG, Espie GS, Fock HP, Canvin DT (1989) Active CO_2 transport by the green alga *Chlamydomonas reinhardtii. Plant Physiology* **89**, 1213–1219.

Sültemeyer DF, Fock HP, Canvin DT (1991) Active uptake of inorganic carbon by *Chlamydonomas reinhardtii*: evidence for simultaneous transport of HCO_3^- and CO_2 and characterization of active CO_2 transport. *Canadian Journal of Botany* **69**, 995–1002.

Sültemeyer DF, Klughammer B, Badger MR, Price GD (1998) Fast induction of high-affinity HCO_3^- transport in cyanobacteria. *Plant Physiology* **116**, 183–192. doi: 10.1104/PP.116.1.183

Tortell PD, Reinfelder JR, Morel FMM (1997) Active uptake of bicarbonate by diatoms. *Nature* **390**, 243–244. doi: 10.1038/36765

Tortell PD, Rau GH, Morel FMM (2000) Inorganic carbon acquisition in coastal Pacific phytoplankton communities. *Limnology and Oceanography* **45**, 1485–1500.

Umino Y, Satoh A, Shiraiwa Y (1991) Factors controlling induction of external carbonic anhydrase and change in $K_{1/2}(CO_2)$ of photosynthesis in *Chlorella regularis. Plant and Cell Physiology* **32**, 379–384.

Williams TG, Colman B (1995) Quantification of the contribution of CO_2, HCO_3^- and external carbonic anhydrase to photosynthesis at low dissolved inorganic carbon in *Chorella* saccharophila. *Plant Physiology* **107**, 245–251.

Young EB, Beardall J, Giordano M (2001) Investigation of inorganic carbon acquisition by *Dunaliella tertiolecta* (Chlorophyta) using inhibitors of putative HCO_3^- utilization pathways. *Europeanal Journal of Phycology* **36**, 81–88.

Manuscript received 23 April 2004, accepted 26 July 2004