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Characterization of diurnal photosynthetic rhythms in the marine diatom *Skeletonema costatum* grown in synchronous culture under ambient and elevated CO_2

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Abstract. Photosynthetic performance was examined in *Skeletonema costatum* (Greville) Cleve. under 12 : 12-h light : dark (LD) cycle at ambient CO_2 (350 μL L^{-1}) and elevated CO_2 (1000 μL L^{-1}). At ambient CO_2, the cellular chlorophyll a content, the light-saturated photosynthetic rate (P_m), the initial slope of the light saturation curves (α), the photochemical efficiency of PSII (F_v/F_m), the apparent carboxylating efficiency (ACE) and the photosynthetic affinity for CO_2 [$1/K_m(CO_2)$] all showed rhythmical changes with different amplitudes during the light period. The P_m had similar changing pattern in the light period with the ACE and $1/K_m(CO_2)$ rather than with the α and F_v/F_m, indicating that rhythmical changes of photosynthetic capacity may be mainly controlled by the activity of C-reduction associated with CO_2 uptake during the light period. The CO_2 enrichment reduced the ACE and the affinity to CO_2, and increased the α, cellular chlorophyll a content and P_m based on cell number. By contrast, the changing patterns of all photosynthetic parameters examined here during the light period had almost the same for cells grown at ambient CO_2 and elevated CO_2, suggesting that the photosynthetic rhythms of *S. costatum* are not affected by CO_2 enrichment.

Keywords: apparent carboxylating efficiency, efficiency of the light reaction, photochemical efficiency of PSII, photosynthetic capacity, photosynthetic CO_2 affinity.

Introduction

Human activities and fossil-fuel burning have led to considerable anthropogenic emissions of carbon dioxide (CO_2). It has been predicted that the atmospheric CO_2 concentration in the end of this century will be 2–3 times that of the present, which may trigger global warming (Wigley and Raper 1992). It is important to assess the physiological and ecological impact of CO_2 enrichment on photosynthesis and growth of aquatic plants (Bowes 1993; Beardall and Giordano 2002). Impacts of CO_2 enrichment on the photosynthetic characteristics have been widely studied in algae. The light-saturated rate of photosynthesis is not affected by CO_2 enrichment in many marine macroalgae (Beer and Koch 1996; Beardall *et al*. 1998) and in some marine microalgae (Muñoz and Merrett 1989; Raven 1997), but most marine embryophytes (i.e. seagrasses) benefit from atmospheric CO_2 enrichment (Beer and Koch 1996; Beardall *et al*. 1998). The photosynthetic affinity for CO_2 and carbonic anhydrase activity in many microalgae declines with CO_2 enrichment (Bowes 1993; Matsuda *et al*. 2002; Colman *et al*. 2002). The photochemical efficiency of PSII increases with the increasing CO_2 in *Chlamydomonas reinhardtii* (Spalding *et al*. 1984).

Marine diatoms, a group of unicellular photosynthetic eukaryotes, are responsible for approximately 40% of marine primary productivity. Considerable research has been carried out on effect of CO_2 enrichment on the growth and photosynthesis of marine diatoms (Colman and Rotatore 1995; Raven and Falkowski 1999; Lane and Morel *et al*. 2000; Burkhardt *et al*. 2001; Morel *et al*. 2002). *Skeletonema costatum* is a unicellular marine diatom that is widely distributed in coastal waters all over the world and constitutes a major component of natural assemblages of most marine phytoplankton. The effects of CO_2 enrichment on diel variation of their growth and photosynthesis have rarely been studied. In this paper, response of diel variation

Abbreviations used: ACE, apparent carboxylating efficiency; CCM, inorganic carbon concentrating mechanisms; DIC, dissolved inorganic carbon; LD, light : dark; P_m, light-saturated photosynthetic rate.

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of photosynthesis during the cell cycle of *S. costatum* to CO_2 enrichment is investigated, which is beneficial to improving our capacity to estimate its gross daily productivity under elevated CO_2.

Diurnal periodicity in photosynthesis has been widely documented to existence in many algae (Senger and Bishop 1967; Sournia 1974; Winter and Brandt 1986; Garczarek *et al*. 2001). The photosynthetic capacity of synchronously grown cells of algae is strongly modulated during the cell cycle, the observed results show the presence of various mechanisms of controlling the photosynthetic capacity during the cell cycle. For example, the C-reduction system has been shown to be the rate-limited step in synchronized cultures of *Euglena gracilis* (Walther and Edmunds 1973). Mishkind *et al*. (1979) showed that the rhythmical variations in photosynthetic activity during the cell cycle of *Ulva* may be modulated by the reoxidation of the plastoquinone pool by the donor for PSI. In addition, the phosphorylation of thylakoid membrane proteins in green algae *Scenedesmus obliquas* (Heil and Senger 1986) and *Chlamydomonas reinhardtii* (Marcus *et al*. 1986) was considered to be correlated with variations of their photosynthetic activity. The experiments presented here also examine the possible mechanism of controlling photosynthetic capacity during the cell cycle of *S. costatum*.

Materials and methods

Skeletonema costatum (Greville) Cleve. (strain 2042) was obtained from the Institute of Oceangraphy, the Chinese Academy of Sciences. Cultures were grown at 20°C and 200 μmol m^{-2} s^{-1} with a12 h light/12 hours dark cycle (12:12 LD cycle) in filtered seawater, which was enriched with 3.0 mM KNO_3, 0.1 mM Na_2HPO_4, 70 μM $NaSiO_3$, 1.0 μM $FeSO_4$ and 25 μM EDTANa. Cultures were aerated with air containing approximately 350 or 1000 μL L^{-1}ppm CO_2. The medium pH ranged from 8.1 to 8.4 and from 7.7 to 7.9 at 350 and 1000 μL L^{-1} CO_2, respectively. Cell density was controlled within a range of 0.5×10^8–3×10^8 cells L^{-1} by dilution every day at the beginning and the end of illumination. Cells at the mid-exponential phase were harvested for the experiments.

Photosynthetic oxygen evolution was measured with a Clark-type oxygen electrode (YSI 5300, YSI incorporated, Yellow Springs, OH). Cells were harvested at the defined time, and were washed and re-suspended in filtered seawater buffered with 20 mM Tris–HCl at pH 8.2 and transferred to electrode chamber and incubated in 5 mL buffer at 20°C.

Photosynthetic parameters for light saturation curves were analysed according to Jassby and Platt (1976):

$$P = P_m \times \tanh\left(\frac{\alpha \times I}{P_m}\right) + R_d,$$

where I is for irradiance; P, for net photosynthetic rate at a given irradiance; P_m, for light-saturated photosynthesis rate; α, for the initial slope of light saturation curve; R_d, for dark respiration. The hyperbolic tangent equation has been shown to give the best fit to field data for coastal assemblages among the various formulations of the light saturation curves (Jassby and Platt 1976; Platt and Jassby 1976), our results showed that it has also good fit to our data.

To determine the dissolved inorganic carbon (DIC)-dependent oxygen evolution, 'CO_2'-free seawater (pH 8.2) was prepared according to Gao *et al*. (1993). Cells were allowed to photosynthesize to deplete possible intracellular pool of 'CO_2' until no net O_2 evolution was observed. Following the addition of $NaHCO_3$ solutions, the rates of oxygen evolution were measured at 400 μmol m^{-2} s^{-1} at 20°C. The K_m DIC or CO_2 values (the DIC or CO_2 concentration required to produce half-maximal photosynthetic rate) were determined according to Michaelis–Menten formula. The apparent carboxylating efficiency (ACE) of Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) was estimated from the initial slope of the DIC-dependent photosynthetic oxygen evolution curves.

Photochemical efficiency of PSII (F_v/F_m) was measured by using a Plant Efficiency Analyzer (PEA, Hansatech instruments Ltd, Pentney, UK). Cells were sampled at defining time intervals, and after acclimation to dark for 10 min tested for F_v/F_m.

Chlorophyll *a* concentration was determined by the spectrophotometric method according to Jeffrey and Humphrey (1975). Cells were counted microscopically by using a haemocytometer.

Results

When *S. costatum* cells were grown in the 12 h light/12 h dark cycles, the cell numbers of the alga grown at the two CO_2 levels in the bulk increased steadily throughout the light period and they were 1.6 and 2.1 times higher after the 12 h light period for the alga grown at 350 and 1000 μL L^{-1} CO_2, respectively (Fig. 1*A*). The chlorophyll *a* concentrations in the bulk of the two CO_2 culture both remained almost constant during the first and the last 2 h of the light period, but they increased 4.4- and 5.4-fold during the middle 8 h of the light period for the alga grown at 350 and 1000 μL L^{-1} CO_2, respectively (Fig. 1*B*). The chlorophyll *a* contents of the two CO_2-grown cells were nearly constant during the first 4 h light period, increased steadily and reached a maximum at 10 h of light period (Fig. 1*C*). The contents of cellular chlorophyll a were higher for the alga grown at 1000 μL L^{-1} CO_2 than that at 350 μL L^{-1} CO_2 (Fig. 1*C*).

Changes of the light-saturated photosynthetic rate, P_m, for the alga grown at the two CO_2 levels during the light period are shown in Fig. 2. The P_m based on chlorophyll *a* content showed clear rhythmical change during the light period, exhibiting a maximal rate at 6 h in the light phase and a minimum at the end of light phase, and there was little difference between P_m of alga grown at low and high CO_2 (Fig. 2*A*). By contrast, P_m based on cell number showed a maximal rate at 8 to 10 h in the light period and a minimum at the beginning of the light period and the rate was higher at high CO_2 than at low CO_2 (Fig. 2*B*).

Variations of the initial slope of the light saturation curve, α, of the alga grown at the two CO_2 levels during the light period were shown in Fig. 3. The values of α reached a maximum and a minimum at the beginning and at the end of the light period, respectively, and the α on average during the light period was lower at the low CO_2 than at the high CO_2 (Fig. 3). The photochemical efficiency of PSII, F_v/F_m, of the alga decreased slightly over the light period, and was slightly higher in the high-CO_2-grown cells than that in the low-CO_2-grown cells (Fig. 4).

The photosynthetic CO_2 affinities, $1/K_m(CO_2)$, of the alga were highest at 6 h of the light period and the CO_2 affinity was markedly higher in cells grown at the low CO_2 than that at the high CO_2 (Fig. 5). Figure 6 showed that the apparent carboxylating efficiencies (ACE) of the alga rhythmically changed and reached a maximum at 3 h of the light period, and the ACE was markedly higher at the low CO_2 than at the high CO_2.

Discussion

Prézelin *et al*. (1977) reported that the photosynthetic pigment (chlorophyll *a* and c_2) contents in marine dinoflagellates *Glenodinium sp.* and *Ceratium furca* were constant over circadian cycles, but Jacquet *et al*. (2001) showed that the chlorophyll contents for three marine *Prochlorococcus spp.* and five marine picoeukaryotes increased throughout the light period and decreased during the dark period. Our results here showed that the chlorophyll *a* contents of *S. costatum* remained almost constant during the first 4 h and increased during the subsequent 6 h culture and then fell in the last 2 h during the light period. The data in Fig. 1 showed that the rate of cell division was relatively constant during the light period (Fig. 1*A*), but the rate of chlorophyll *a* synthesis fluctuated (Fig. 1*B*) and the rate was, on average, higher than that of cell division during the light period. Thus, the variation of the chlorophyll *a* content during the light period largely resulted from the fluctuating ratio of the rate of the chlorophyll *a* synthesis *v.* the cell division in *S. costatum*. The variation of the chlorophyll a content resulted in a difference in the pattern of P_m between that based on chlorophyll *a* content and that based on cell number during the light period.

Rhythmical change of photosynthetic capacity, P_m, during the cell cycle has been documented for several

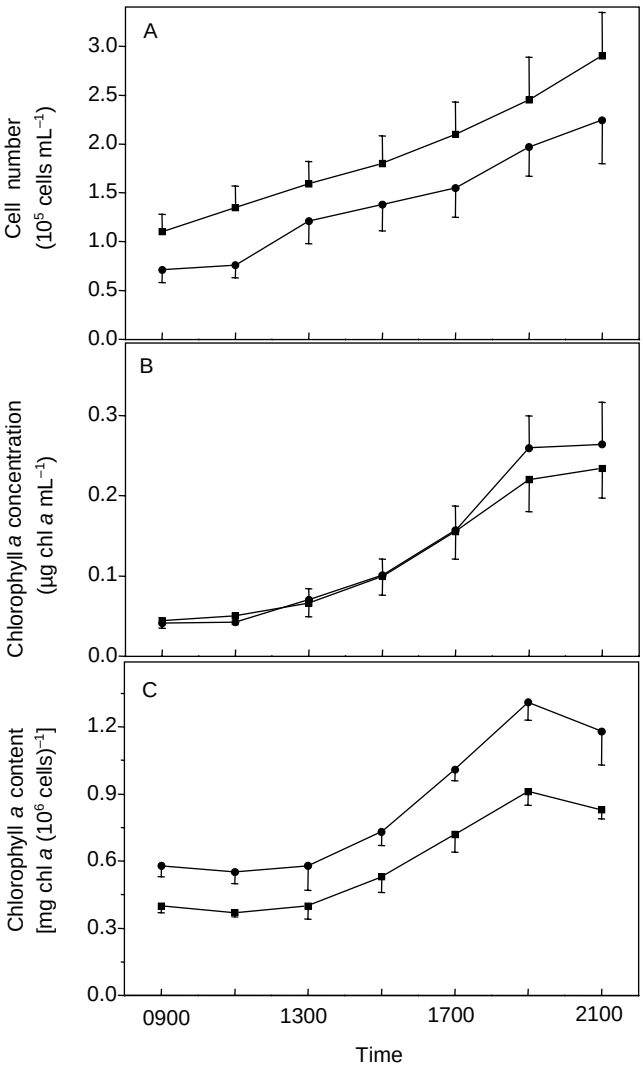

Fig. 1. Changes of (*A*) cell number and (*B*) chlorophyll *a* concentration in the bulk and (*C*) chlorophyll *a* content of cell for *Skeletonema costatum* cells grown at 200 μmol m^{-2} s^{-1} and 350 (■) or 1000 (●) μL L^{-1} CO_2 during the light period. Values are mean ± s.e. of three separate experiments.

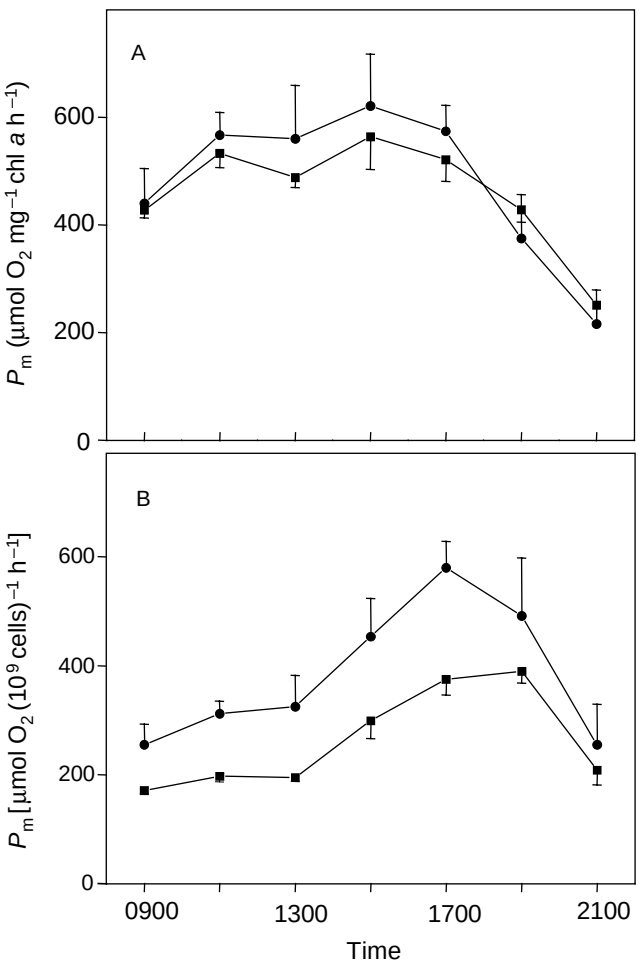

Fig. 2. Changes of light-saturated photosynthetic rate, P_m, of *Skeletonema costatum* cultured at 200 μmol m^{-2} s^{-1} and 350 (■) or 1000 (●) μL L^{-1} CO_2 during the light period, (*A*) based on cellular chlorophyll a content, (*B*) based on cell number. Values are mean ± s.e. of three separate experiments.

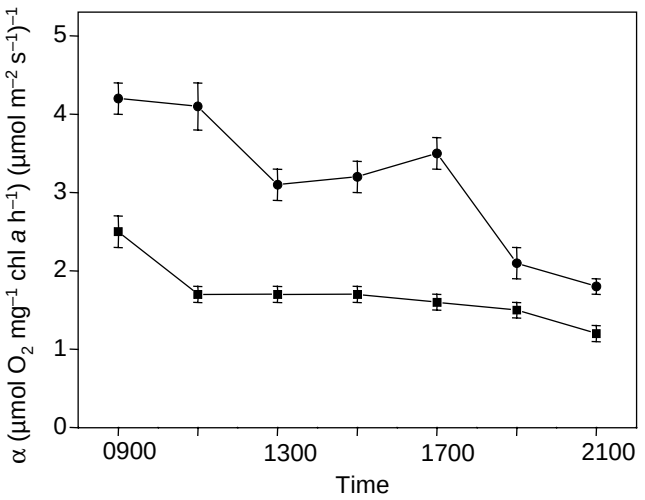

Fig. 3. Changes of the initial slope of the light saturation curve, α, of *Skeletonema costatum* cultured at 200 μmol m^{-2} s^{-1} and 350 (■) or 1000 (●) μL L^{-1} CO_2 during the light period. Values are mean ± s.e. of three separate experiments.

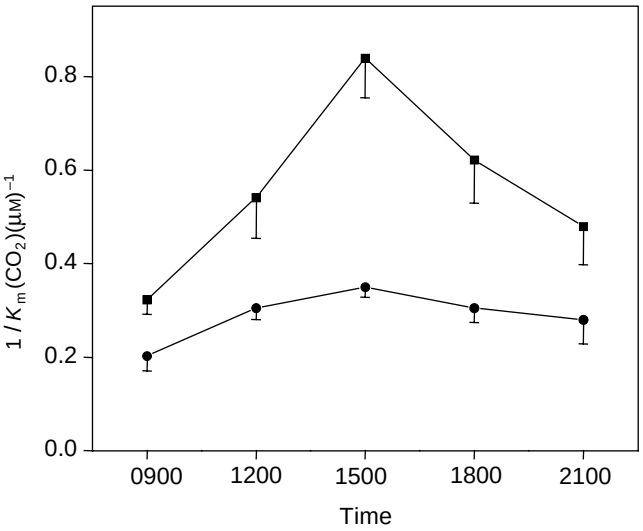

Fig. 5. Changes of the photosynthetic CO_2 affinity, $1/K_m(CO_2)$, of *Skeletonema costatum* cultured at 200 μmol m^{-2} s^{-1} and 350 (■) or 1000 (●) μL L^{-1} CO_2 during the light period. Values are mean ± s.e. of three separate experiments.

groups of alga and mixed populations of phytoplankton (MacCaull and Platt 1977; Prézelin *et al*. 1977; Mishkind *et al*. 1979; Kaftan *et al*. 1999), the time at which P_m is maximal varies but is most often in the morning or midday. Our data showed that the P_m of *S. costatum* varied rhythmically during the light period; the maximal P_m peaked in the midday when expressed on chlorophyll a but in the afternoon when expressed on the basis of cell number. The differences may be due to the diel variation of chlorophyll *a* content.

The factors that control photosynthetic activity during the cell cycle have not been identified. Some studies indicate that PSII activity and phosphorylation of thylakoid protein may play a role in controlling the change of the photosynthetic activity (Marcus *et al*. 1986; Allen 1992; Kaftan *et al*. 1999), and that the diel pattern of photosynthetic activity is directly correlated with the initial slope of the light-saturation curve (Harding *et al*. 1987; Erga and Skjoldal 1990), whereas other studies point out that C-reduction may be the rate-limiting factor (Walther and Edmunds 1973; Myers and Graham 1975). In the present study, the changing pattern of the P_m of *S. costatum* during

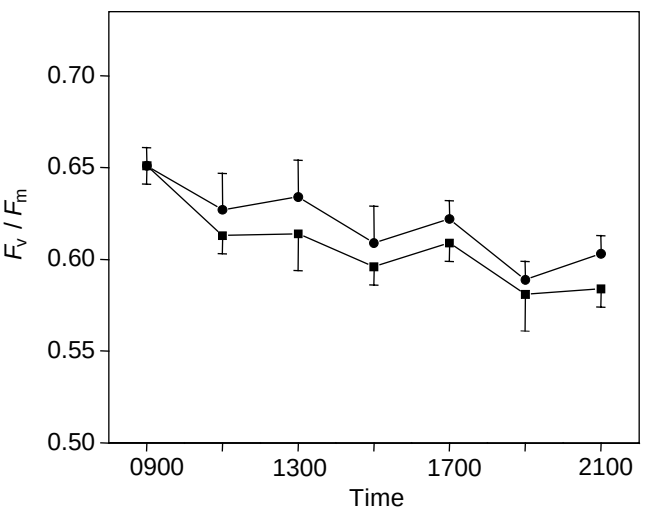

Fig. 4. Changes of the photochemical efficiency of PSII, F_v/F_m, of *Skeletonema costatum* cultured at 200 μmol m^{-2} s^{-1} and 350 (■) or 1000 (●) μL L^{-1} CO_2 during the light period. Values are mean ± s.e. of three separate experiments, $n = 9$.

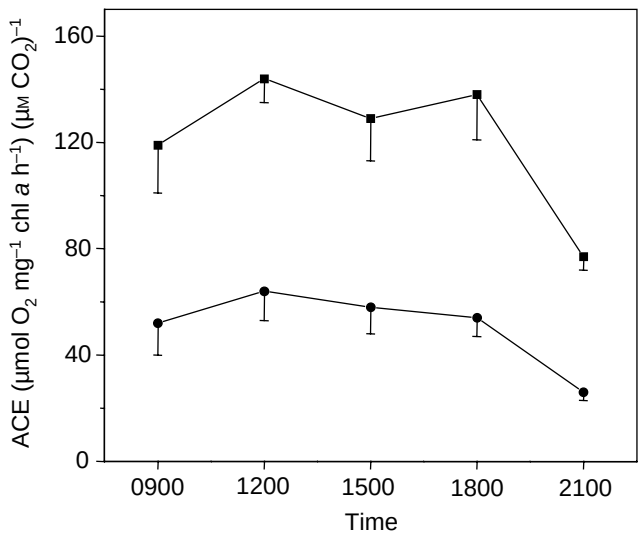

Fig. 6. Changes of the apparent carboxylating efficiency (ACE) of *Skeletonema costatum* cultured at 200 μmol m^{-2} s^{-1} and 350 (■) or 1000 (●) μL L^{-1} CO_2 during the light period. Values are mean ± s.e. of three separate experiments.

the light period was proportional to that of apparent carboxylating efficiency and that of photosynthetic CO_2 affinity, but was not correlated with that of the initial slop of the light saturation curve (α), which is indicative of the efficiency of the light reaction, and that of the photosynthetic chemistry efficiency of PSII (F_v/F_m). These results indicated that the changing pattern of the photosynthetic capacity during the light period might be controlled by the activity of C-reduction associated with C-uptake in *S. costatum* in dark reaction. *Skeletonema costatum* has been reported to have the capacity to actively take up both CO_2 and HCO_3^- (Korb *et al*. 1997). Our experiments showed that the alga mainly use free CO_2 from the medium (unpublished data), suggesting that the daily changes in the photosynthetic CO_2 affinity may be attributed to the diurnal alterations of photosynthetic capacity during the light period.

The results presented in this paper showed that the P_m based on chlorophyll *a* was not significantly different between ambient CO_2 and enriched CO_2, whereas the P_m based on cell number increased with CO_2 enrichment (because of the increased chlorophyll *a* content per cell under enriched CO_2). The result indicated that the estimation on effect of CO_2 enrichment on photosynthetic capacity was related to the normalized units, and implied that *S. costatum* benefited from CO_2 enrichment. The α and F_v/F_m increased with increasing CO_2, indicating that the efficiency of light-harvesting and energy conversion in photosynthesis were increased. Down-regulation of Rubisco and inorganic carbon concentrating mechanisms (CCM) activities has been reported in many species (Wong 1979; Vu *et al*. 1983; Bowes 1993; Colman 2002; Matsuda *et al*. 2002). The present study indicated that the apparent carboxylating efficiency and the photosynthetic affinity for CO_2 markedly decreased under elevated CO_2 in *S. costatum*, suggesting that the similar down-regulation of Rubisco and CCM activities may also exist in this alga.

The effects of elevated CO_2 on photosynthetic rhythms during the cell cycle have been studied. Nara *et al*. (1989) reported that the changing patterns of the photosynthetic activity and the CA activity and the affinity of cell for CO_2 of *Chlorella ellipsoidea* C-27 were almost the same for cells synchronized at high (700 μM) and low (11 μM) levels of CO_2. Our data showed that the P_m based on cell number, the chlorophyll *a* content, the photosynthetic chemistry of PSII and the efficiency of the light reaction all increased to various degrees with elevated CO_2 and that the apparent carboxylating efficiency and the photosynthetic affinity for CO_2 markedly decreased under high CO_2 concentration in *S. costatum*. However, the changing patterns of all these photosynthetic parameters during the light period were almost the same for *S. costatum* cells grown at high and low levels of CO_2, suggesting that the photosynthetic rhythms was insensitive to CO_2 enrichment.

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