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Oxygen enhancement of photosynthetic $^{14}CO_2$ fixation in a freshwater diatom *Nitzschia ruttneri*

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Oxygen enhanced the rate of photosynthetic $^{14}CO_2$ fixation in a freshwater diatom *Nitzschia ruttneri* Hust. The rate under 21% O_2 (79% N_2) was 20 to 35% higher than that under 0% O_2 (100% N_2), and even under 100% O_2, the rate was almost the same or rather higher than that under 0% O_2. The response to oxygen similar to that in total photosynthesis rate was observed only in ^{14}C-incorporation into β-1,3-glucans during photosynthesis. The photosynthetic response to oxygen concentrations was not affected by the CO_2 concentration during photosynthesis in the presence and absence of 0.1 mM acetazolamide (AZA), a carbonic anhydrase inhibitor, although AZA decreased the photosynthetic affinity for CO_2, increasing $K_{1/2}$ for CO_2 from about 2 to 9 μM under 21% O_2. Very high carbonic anhydrase activity, which was mostly extracytoplasmic, was observed only when the cells were adapted to air. ^{14}C-incorporation into glycolate during photosynthetic $^{14}CO_2$ fixation was negligible under 21% O_2 and quite low even under 100% O_2 in spite of the C_3-plant type ^{14}C-labelling pattern of photosynthetic products. The oxygen enhancement of CO_2 fixation in this diatom does not seem to be related to "CO_2 concentrating mechanism" but to photosynthetic electron flow such as the Mehler reaction.

Key Index Words: carbonic anhydrase—CO_2 concentrating mechanism—Nitzschia—*oxygen effect—photorespiration—photosynthesis.*

Photosynthetic CO_2 fixation in terrestrial C_3 plants is inhibited competitively by oxygen when CO_2 concentration is rate-limiting; by 30 to 50% even under atmospheric conditions (21% O_2, 0.035% CO_2). Such oxygen inhibition is caused mainly by photorespiration derived from the oxygenase activity of RuBP carboxylase/oxygenase (Rubisco).

In algae, different types of oxygen effects on photosynthesis have been reported. Unicellular algae such as *Chlamydomonas reinhardtii* show almost no oxygen inhibition of photosynthesis when grown under limiting-CO_2 conditions (Lloyd *et al.* 1977, Spalding *et al.* 1983a, b, Suzuki and Spalding 1989a, b). In air-grown cells of *Chlorella pyrenoidosa*, oxygen inhibition was observed but was not sensitive to CO_2 (Shelp and Canvin 1980, 1981). The lack of CO_2-sensitive O_2 inhibition in these algae has been explained by the operation of a CO_2 concentrating mechanism in air-adapted cells (Badger *et al.* 1980) which eliminates photorespiration by raising the CO_2/O_2 ratio at the site of Rubisco and traps any released photorespiratory CO_2 very efficiently (Suzuki and Spalding 1989b).

It has been reported in the cyanobacterium *Anacystis nidulans* that oxygen enhanced the rate of photosynthetic $^{14}CO_2$ fixation (Miyachi and Okabe 1976). The enhancement was observed only under CO_2-limiting conditions and was highest under 10% O_2. They proposed that oxygen is necessary for providing the CO_2 supply to the site of Rubisco in this alga, but no evidence has been presented for an oxygen requirement for the CO_2-concentrating mechanism in any algal species.

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[2] Abbreviations: AZA, acetazolamide; RuBP, ribulose-1,5-bisphosphate.

Oxygen enhancement of photosynthetic $^{14}CO_2$ fixation has been also observed in a cryptomonad, *Chroomonas* sp. (Suzuki and Ikawa 1984a, b, 1985). The oxygen enhancement, however, was not affected by CO_2 concentration in this alga but was related to the oxygen requirement of the photosystems under light-saturating conditions to overcome over-reduction. The excessive electrons produced in *Chroomonas* cells during photosynthesis under light-saturating conditions seems to be removed from photosystem I by the Mehler reaction to keep adequate levels of ATP and NADPH for CO_2 fixation (Suzuki and Ikawa 1984a, b, 1985). The oxygen enhancement was saturated under 2% O_2, and under higher oxygen concentrations photosynthesis in *Chroomonas* sp. showed a CO_2-insensitive inhibition (Suzuki and Ikawa 1984a, 1985) similar to that reported in *C. pyrenoidosa*.

In this paper, we report an oxygen enhancement of photosynthetic $^{14}CO_2$ fixation in a freshwater diatom *Nitzschia ruttneri*, which appears to have a different cause from that in *Anacystis nidulans* or *Chroomonas* sp.

Material and Methods

The freshwater diatom *Nitzschia ruttneri* Hust. (NT-1A) is a gift from Dr. Isao Inouye, Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan. The cells were grown axenically in 1-liter Sakaguchi flasks containing 500 m*l* of a liquid medium modified from the FWT medium (Darley and Volcani 1971), which has the following composition: $Ca(NO_3)_2 \cdot 4H_2O$, 0.15 g; K_2HPO_4, 0.05 g; $MgSO_4$, 0.025 g; $Na_2SiO_4 \cdot 9H_2O$, 0.5 g; Darley and Volcani's trace elements, 1 m*l*; Bacto-tryptone, 0.5 g; glycylglycine, 0.5 g; in 1 liter of distilled water, pH 7.6. The cells were illuminated with cool-white fluorescent tubes at an intensity of about 12–15 $W \cdot m^{-2}$ at the culture flask level, using 12-h light 12-h dark cycle, and bubbled continuously with filtered air without supplementary CO_2, at 18°C.

Cells in the late log phase of growth (6–7 days old) were harvested by centrifugation at 200 × g for 5 min and resuspended in 20 mM HEPES-KOH buffer, pH 7.6 (containing the inorganic components of the growth medium).

Photosynthetic $^{14}CO_2$ fixation experiment and the analysis of products were performed as described previously (Suzuki and Ikawa 1984a, 1985). One ml of cell suspension (about 10 μg Chl) was placed in spitz-type test tube (16 × 150 mm) at 20°C. CO_2-free gas mixtures with known ratios of O_2/N_2 were bubbled at 120 m$l \cdot min^{-1}$ through the algal suspension during the experiments. After 10 min-illumination with a halogen lamp at 200 $W \cdot m^{-1}$, photosynthetic CO_2 fixation was started by injecting $NaH^{14}CO_3$ and stopped 5 min later by adding boiling ethanol to a concentration of 80% (v/v). For time-course experiments, 6 m*l* of algal suspension was placed in a test tube. After injecting $NaHCO_3$, 1-m*l* aliquots of algal suspension were removed at intervals and put into a test tube containing 4 m*l* of boiling ethanol. Analysis of $^{14}CO_2$-fixation products was performed using two-dimensional paper chromatography and the subsequent radioautography (Suzuki and Ikawa 1984a, 1985).

Carbonic anhydrase assays were performed by monitoring the pH change at 2°C in 25 mM barbital-buffered solution (Spalding and Ogren 1982). Enzyme units were calculated from the equation: $U = t_b/t_s - 1$, where t_b and t_s represent the time (sec) measured for the pH change (8.0 to 7.5) with buffer alone (t_b) and with sample (t_s) (Suzuki and Spalding 1989b).

Chlorophyll was determined after extraction into 80% (v/v) ethanol (Suzuki and Ikawa 1984a).

Results

Oxygen effect on the rate of photosynthesis

Figure 1 shows the relative rate of photosynthetic $^{14}CO_2$ fixation in a diatom *Nitzschia ruttneri* under different concentrations of oxygen. The photosynthesis rate was highest under 21% O_2 and was higher by 20 to 35%

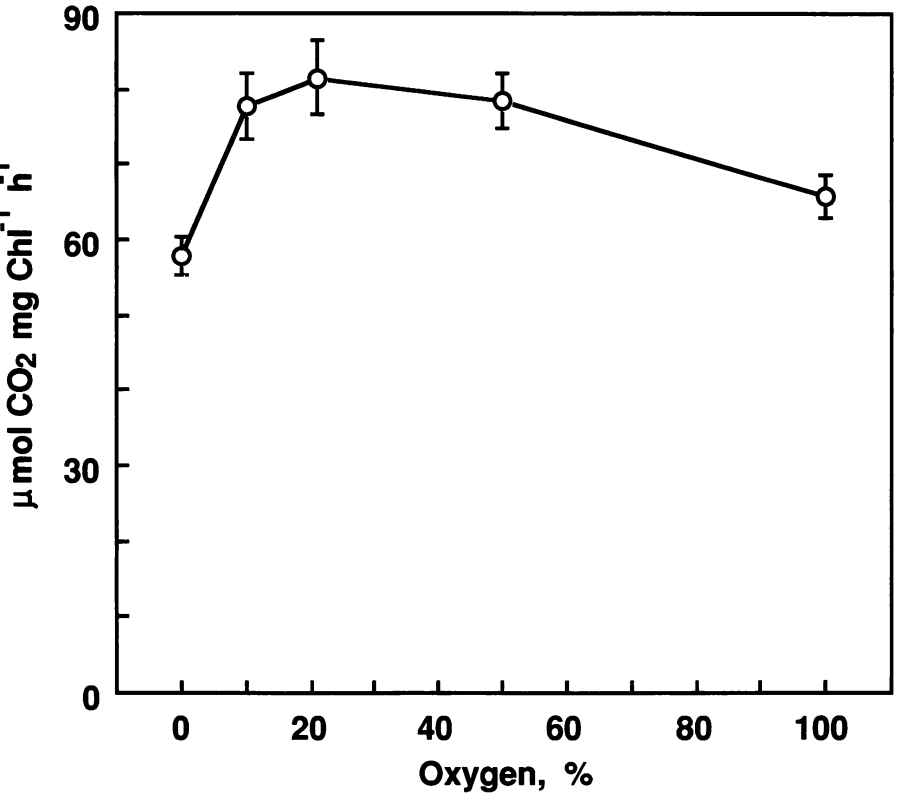

Fig. 1. Effect of oxygen on photosynthetic $^{14}CO_2$ fixation at 0.7 mM $NaHCO_3$. The rate under 21% O_2, 81.5±20.8 μmol CO_2 mg Chl^{-1} h^{-1}, was expressed as 100% and the relative rate under other O_2 concentrations were the means±SD (vertical bars) of 19 independent experiments.

than that under 0% O_2. The rate under 100% O_2 was 10 to 30% lower than that under 21% O_2, and was almost the same or slightly higher than that under 0% O_2 in most of the experiments. This phenomenon, the oxygen enhancement of photosynthesis, appears similar to that reported in *Anacystis nidulans*, where the photosynthetic maximum was at around 10% O_2 (Miyachi and Okabe 1976). The oxygen enhancement in *A. nidulans* was observed only under CO_2-limiting conditions. In *N. ruttneri*, however, the oxygen enhancement was observed at all $NaHCO_3$ concentrations tested and the response of photosynthesis to oxygen was not affected by $NaHCO_3$ concentration at least within the range from 0.08 to 1.7 mM (pH 7.6) (Fig. 2). This CO_2 insensitivity in the oxygen effect is very similar to that in *Chroomonas*. sp., although photosynthesis was highest under 2% O_2 in *Chroomonas* (Suzuki and Ikawa 1984a). The $K_{1/2}$ of photosynthetic $^{14}CO_2$ fixation for $NaHCO_3$ was lower than 50 μM (about 2 μM CO_2 at pH 7.6) under 0% O_2, 21% O_2 and 100% O_2 in *N. ruttneri* (Fig. 2), which is very close to that of *Chroomonas* sp. (Suzuki and Ikawa 1984a) or that of *Chlamydomonas reinhardtii* (Suzuki and Spalding 1989b).

^{14}C-labelling pattern during photosynthetic $^{14}CO_2$ fixation

Figure 3 shows the percentage ^{14}C-incorporation into individual products in the 80% ethanol soluble fraction during photosynthetic $^{14}CO_2$ fixation under 21% O_2 at 0.66 mM $NaHCO_3$ (33 μM CO_2 at pH 7.6). About 85% was incorporated into 3-phosphoglycerate, and more than 90% was in phosphate esters, after 12 sec of $^{14}CO_2$ fixation. The percentage incorporation into 3-phosphoglycer-

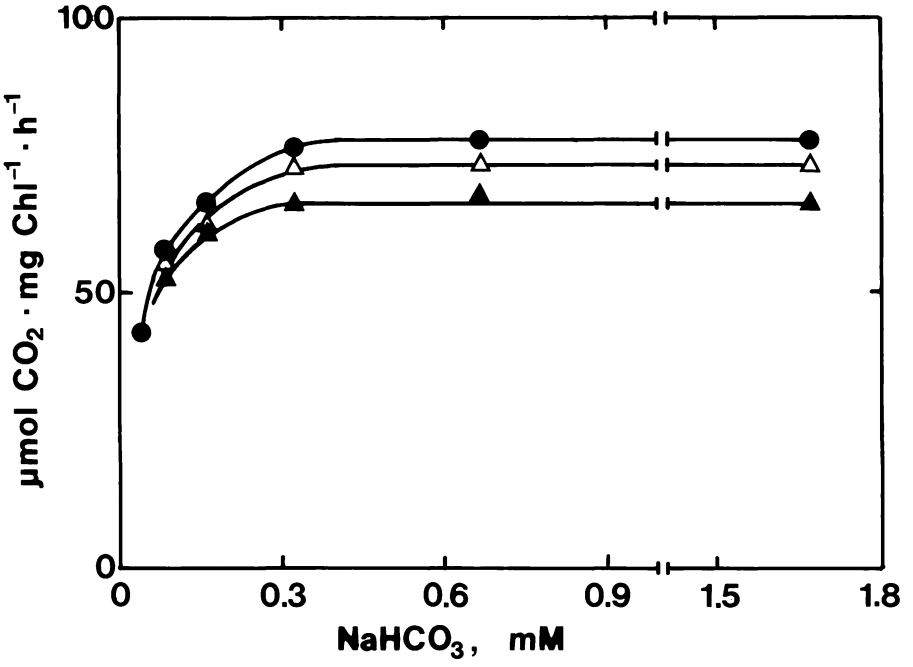

Fig. 2. Effect of oxygen on photosynthetic $^{14}CO_2$ fixation at various $NaHCO_3$ concentrations. ▲, 0% O_2; ●, 21% O_2; △, 100% O_2.

ate decreased with increasing time. No such negative curve was observed for ^{14}C-incorporation into other products, and no more than 6% of ^{14}C in the soluble fraction was found in C_4 acids, aspartate and malate, after 12 sec. These results suggest that 3-phosphoglycerate is the initial stable product of photosynthetic CO_2 fixation and that the photosynthetic pathway in this diatom is very similar to that of C_3 plants. However, ^{14}C-incorporation into the products involved in photorespiration, such as glycolate, glycine and serine, was very low during 10-min photosynthesis under 21% O_2.

Total ^{14}C incorporated into acid-stable products increased almost linearly for 10 min after $NaH^{14}CO_3$ addition, but a large portion (about 74%) of the ^{14}C fixed was found in the 80% ethanol-insoluble fraction after 10 min; whereas only about 30% was found after 1 min (data not shown). Preliminary studies using β-1,3-glucanase showed that most of ^{14}C in the insoluble fraction seems to be incorporated into β-1,3-glucans, at least during 5 min of $^{14}CO_2$ fixation (data not shown).

Effect of oxygen on ^{14}C distribution among the products

Table 1 shows the effect of oxygen on ^{14}C-distribution among individual products during photosynthetic $^{14}CO_2$ fixation. Percent ^{14}C-incorporation into glycolate, glycine and serine, which are photorespiratory products in green plants, was very low, and that in glycine and serine did not increase with increasing oxygen, although that in glycolate was increased slightly. Percentage in glycine and serine, on the other hand, decreased with increasing oxygen. The major part of ^{14}C in glycine and serine seems not to come from photorespiration. Instead, percentages of ^{14}C in glutamate and aspartate were increased with increasing oxygen.

Percentage of ^{14}C in 3-phosphoglycerate decreased with increasing oxygen, while the other phosphate esters showed no significant change. Relative ^{14}C-incorporation into the 80% ethanol-insoluble fraction, mostly β-1,3-glucans (so-called crysolaminalin), was maximal under 21% O_2 where the photosynthetic rate was highest. However, percentage in

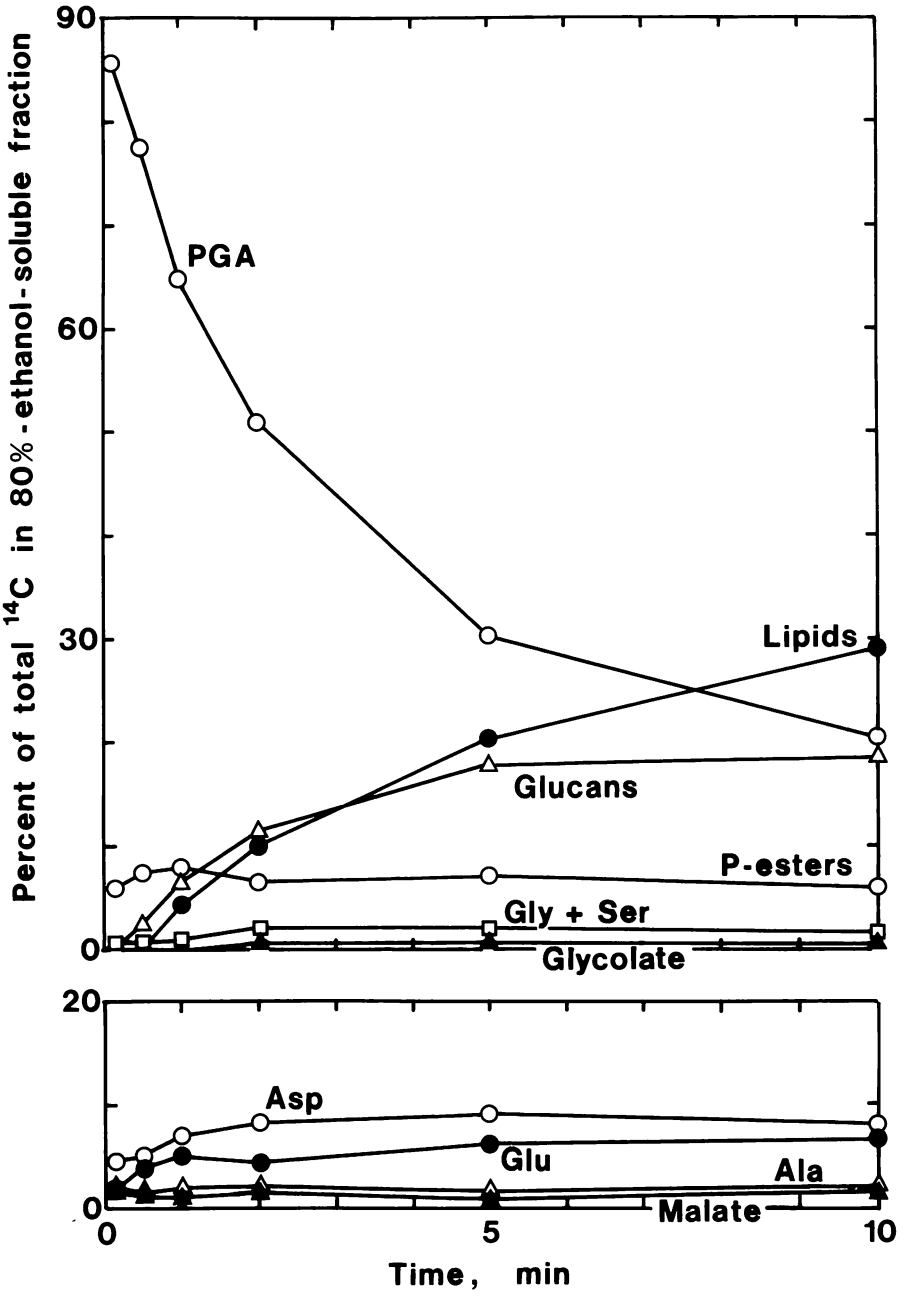

Fig. 3. Percentage distribution of ^{14}C in individual products of 80% ethanol-soluble fraction *versus* time of photosynthetic $^{14}CO_2$ fixation under 21% O_2 at 0.7 mM $NaHCO_3$. Glucans, β-1,3-glucans; P-esters, phosphate esters other than PGA; PGA, 3-phosphoglycerate.

the soluble glucans was highest under 0% O_2 and decreased with increasing oxygen.

The relatively high ^{14}C-incorporation into 3-phosphoglycerate and soluble glucans under 0% O_2 suggests that the cause of oxygen enhancement is related to energy supply. However, an accumulation of ^{14}C-triose phosphate under anaerobic condition (Suzuki and Ikawa 1985) was not observed.

Table 1. Effect of oxygen on distribution of ^{14}C among the products after 5-min of photosynthetic $^{14}CO_2$ fixation in *Nitzschia ruttneri* at 0.7 mM $NaHCO_3$, pH 7.6 and 20°C. Chl content in the cell suspension was 12 $\mu g \cdot ml^{-1}$.

product	O_2 concentration in bubbling gas					
	0%		21%		100%	
	amount[a]	%	amount[a]	%	amount[a]	%
(origin)	0.2	0.0	0.2	0.0	0.1	0.0
phosphate esters	79.0	20.7	99.9	15.6	77.4	13.5
3-PGA[b]	65.3	17.1	81.3	12.7	56.7	9.9
others	13.8	3.6	18.6	2.9	20.6	3.6
aspartate	12.3	3.2	24.3	3.8	37.2	6.5
glutamate	6.9	1.8	16.6	2.6	49.3	8.6
Gly+Ser[c]	4.0	1.0	5.1	0.8	4.8	0.8
citrate	0.4	0.1	0.3	0.0	1.1	0.2
malate	1.5	0.4	4.5	0.7	4.6	0.8
glycolate	0.0	0.0	1.3	0.2	8.0	1.4
glutamine	0.0	0.0	0.6	0.1	2.3	0.4
alanine	2.9	0.8	3.8	0.6	5.7	1.0
lipids	42.8	11.2	54.4	8.5	74.5	13.0
s.glucans[d]	34.8	9.1	48.0	7.5	26.4	4.6
insoluble[e]	175.6	46.0	369.4	57.7	272.2	47.5
others	21.4	5.6	11.8	1.8	9.7	1.7
total	381.7	(100)	640.2[f]	(100)	573.3	(100)

[a] $dpm \cdot ng\ Chl^{-1}$; [b] 3-phosphoglycerate; [c] glycine and serine; [d] β-1,3-glucans in 80% ethanol-soluble fraction; [e] 80% ethanol-insoluble fraction; [f] 62.9 $\mu mol \cdot mg\ Chl^{-1} \cdot h^{-1}$.

Carbonic anhydrase

When the cells were grown under air, very high carbonic anhydrase activity was observed in the intact cell suspension and the sonicate (Table 2). The activity from the intact cells was at least 83% of that from the sonicate, suggesting carbonic anhydrase in *N. ruttneri* is mostly extracytoplasmic as reported in *Chlamydomonas reinhardtii* (Kimpel *et al.* 1983). The activity was inhibited 90% by 0.1 mM AZA (Table 2) and the K_i value was about 5 nM (data not shown). About 75% of the activity was lost by adaptation to 3% CO_2 for 24 h and mostly lost after 6-day growth

Table 2. Carbonic anhydrase activity in the different enzyme preparations from *Nitzschia ruttneri*. Number in parenthesis is the standard error (n=3).

Cell type	Preparation	Carbonic anhydrase activity (units[a]/mg Chl in 3.5 ml)	
Air-grown cells[b]	intact cells	386.0	(±4.7)
	medium	0[e]	
	sonicate	465.1	(±7.8)
	sonicate+0.1 mM AZA	33.4	(±2.4)
24-h 3% CO_2-adapted cells[c]	sonicate	60.0	(±4.0)
3% CO_2-grown cells[d]	sonicate	3.0	(±1.1)

[a] $t_b/t_s - 1$, where t_b and t_s represent the time (sec) measured for the pH change (8.3–7.3) with 25 mM barbital buffer alone (t_b) and with sample (t_s) at 2°C.
[b] grown with aeration by air (ca. 0.04% CO_2).
[c] aerated with air supplemented with 3% CO_2 for 24 h prior to use after grown under air.
[d] grown for 6 days with aeration by air supplemented with 3% CO_2 after grown under air.
[e] not detected.

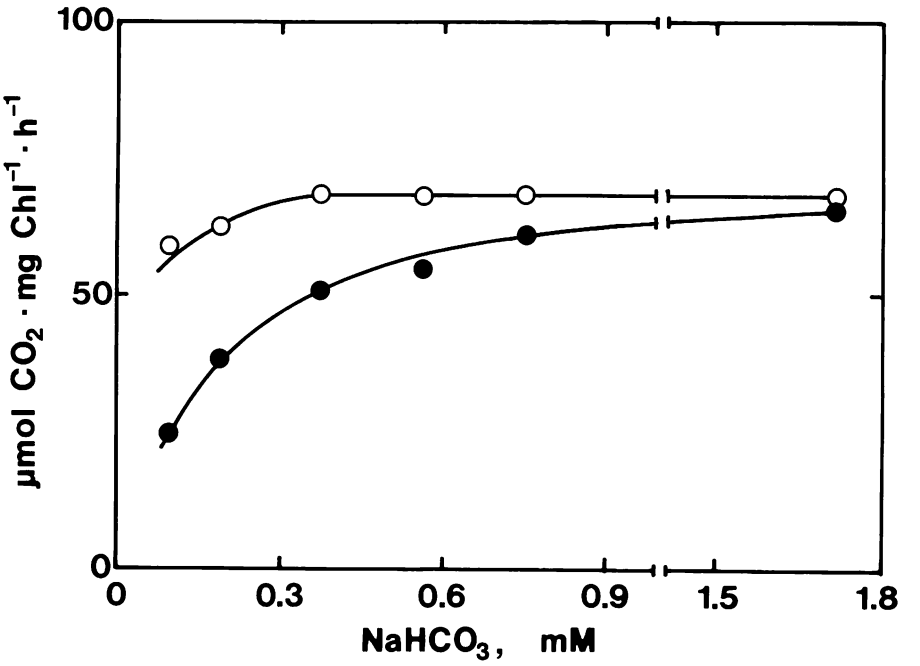

Fig. 4. Effect of 0.1 mM acetazolamide on photosynthetic $^{14}CO_2$ fixation under 21% O_2 at various $NaHCO_3$ concentrations. ○, control; ●, +0.1 mM acetazolamide. Chl content was 7.4 $\mu g\ ml^{-1}$.

under 3% CO_2 (Table 2).

Effect of AZA on photosynthetic $^{14}CO_2$ fixation

The rate of photosynthetic $^{14}CO_2$ fixation in *N. ruttneri* was inhibited only slightly by the addition of 0.1 mM AZA at 1.8 mM $NaHCO_3$ (Fig. 4). The inhibition by AZA, however, increased with decreasing $NaHCO_3$ concentration, causing a high $K_{1/2}$ value of photosynthesis for $NaHCO_3$; about 150 μM, compared to 50 μM without AZA. This suggests that an extracytoplasmic carbonic anhydrase plays an important role in an inorganic carbon utilizing mechanism in this diatom as proposed in some unicellular algae (Imamura *et al.* 1983, Tsuzuki 1983, Marcus *et al.* 1984, Moroney and Tolbert 1985, Moroney *et al.* 1985, Aizawa and Miyachi 1984, Sültemeyer *et al.* 1989). However, the addition of 0.1 mM AZA did not affect the photosynthetic response to oxygen under either 0.095 or 0.75 mM $NaHCO_3$ (Fig. 5).

Discussion

Oxygen enhancement of photosynthesis

It was proposed that the oxygen enhancement of photosynthesis in the cyanobacterium *Anacystis nidulans* was related to an oxygen requirement for a CO_2 concentrating mechanism, based on the fact that the oxygen enhancement was observed only under CO_2-limiting conditions without significant effect on ^{14}C-distribution among the products (Miyachi and Okabe 1976). So far in algae, however, there is no evidence for an oxygen requirement for CO_2 concentrating mechanisms.

We also observed an oxygen enhancement of photosynthetic $^{14}CO_2$ fixation in a freshwater diatom *Nitzschia ruttneri*, with the enhancement being greatest under 21% O_2 (Fig. 1). This diatom has characteristics which strongly suggest the operation of a CO_2-concentrating mechanism when adapted to air, such as a very high affinity of photosynthesis for CO_2 in air-grown cells (Fig. 2), sensitivity to AZA (Fig. 4), and a high ex-

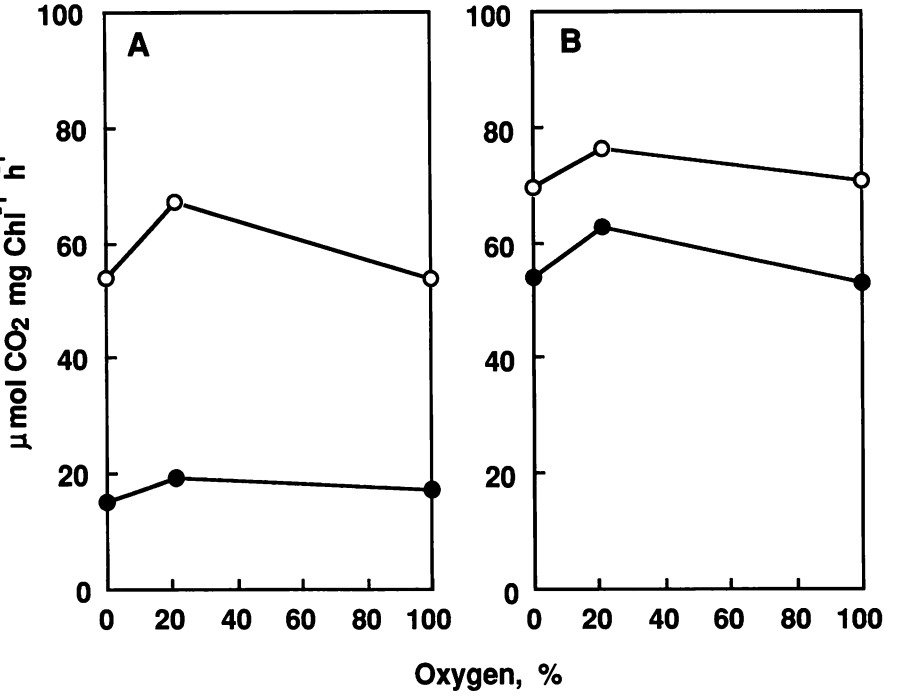

Fig. 5. Effect of acetazolamide on photosynthetic $^{14}CO_2$ fixation at various O_2 concentrations at 0.095 mM (A) and 0.75 mM (B) $NaHCO_3$. ○, control; ●, +0.1 mM acetazolamide. Chl content was 7.2 $\mu g\ ml^{-1}$.

tracytoplasmic activity of carbonic anhydrase observed only in air-grown or air-adapted cells (Table 2). The mechanism seems to be similar to that proposed in unicellular freshwater green algae such as *C. reinhardtii* (Badger *et al.* 1980, Suzuki and Spalding 1989b, Suzuki *et al.* 1990), rather than that proposed in the marine diatom *Phaeodactylum tricornutum* in which extracytoplasmic carbonic anhydrase was not detected and AZA did not affect the photosynthetic affinity for CO_2 (Patel and Merrett 1986, Dixon and Merrett 1988). Thus, it seems unlikely that the mechanism proposed in *P. tricornutum* occurs generally in the diatoms, but it is still not clear if the differences between the two diatoms come from the difference in their habitats, freshwater or marine, or not.

It seems unlikely that oxygen stimulates the inorganic carbon utilizing mechanism in *Nitzschia ruttneri* as proposed in *A. nidulans* (Miyachi and Okabe 1976), because the oxygen effect in *N. ruttneri* was not sensitive to CO_2 concentration (Fig. 2) even in the presence of AZA (Fig. 5). The cause of the oxygen enhancement in *N. ruttneri* may be rather similar to that in *Chroomonas* sp, although the enhancement in *N. ruttneri* appears to require higher O_2 concentration.

In *Chroomonas*, when exposed to light-saturating conditions, photosynthetic CO_2 fixation under 0% O_2 was inhibited by ATP deficiency which was derived from an over-reduction of photosynthetic electron transport via the inhibition of both cyclic and pseudocyclic photophosphorylation (Suzuki and Ikawa 1984b, 1985). The accumulation of ^{14}C-dihydroxyacetone phosphate observed during photosynthetic $^{14}CO_2$ fixation in *Chroomonas* under 0% O_2 (Suzuki and Ikawa 1985) can be explained as an indirect consequence of the ATP deficiency. The high level of inorganic phosphate caused by ATP deficiency should activate the phosphate translocator across the chloroplast envelope, which, in *Chroomonas* cells, might cause an excessive efflux of dihydroxyacetone phosphate to cytozol where the accumulation should oc-

cur. Thus, while the accumulation of dihydroxyacetone phosphate was not observed in *N. ruttneri* (Table 1), it is possible to explain the oxygen enhancement of photosynthesis in *N. ruttneri* by a limitation in the ATP supply, partly because ^{14}C-incorporation into β-1,3-glucans, which is supposed to require a significant level of ATP supply, was affected by oxygen almost in the same manner as total $^{14}CO_2$ fixation rate while the percentage incorporation of ^{14}C into PGA was the highest under N_2 (Table 1). Our preliminary work also suggested an overreduction of photosynthetic electron flow under 0% O_2, in which the oxygen enhancement in *N. ruttneri* disappeared in the presence of DCMU (data not shown). Further studies are necessary to confirm the cause of the oxygen enhancement of photosynthesis in *N. ruttneri*.

Photorespiration and oxygen inhibition of photosynthesis

During 5 min of photosynthesis under 21% O_2, almost no glycolate formation was observed in air-grown cells of *N. ruttneri* (Table 1), as reported in some other algae such as *Chroomonas* sp. (Suzuki and Ikawa 1985) and *Chlamydomonas reinhardtii* (Suzuki *et al.* 1990). In *Chlamydomonas* grown under 5% CO_2, when a CO_2 concentrating mechanism was not operational, not less than 6% of ^{14}C fixed was incorporated into glycolate (Suzuki *et al.* 1990). It is unlikely that the quite low level of ^{14}C-incorporation into glycolate is due to a high turn-over rate of glycolate metabolism, because the activity of glycolate dehydrogenase in the crude extract from this diatom was only about 5 μmol DCPIP reduction $\cdot$ mg $Chl^{-1} \cdot h^{-1}$ at 8 mM glycolate, and the K_m value for glycolate was much lower than those in green algae (data not shown; ref. Paul and Volcani 1974, Suzuki *et al.* 1991). Thus, while photosynthesis in *N. ruttneri* is inhibited by increasing oxygen concentration from 21 to 100% (Fig. 1), it does not seem to be caused by photorespiration but by a reaction such as the Mehlar reaction as suggested in *Chroomonas* sp. (Suzuki and Ikawa 1984a, 1985). Glycolate synthesis seems to be strongly suppressed in this diatom even under extremely high O_2 concentrations, probably by a CO_2 concentrating mechanism as proposed in *Chlamydomonas* in which the suppression of photorespiration is due to the raised CO_2/O_2 ratio at the site of RuBP carboxylase/oxygenase (Badger *et al.* 1980). However, as the accumulation of inorganic carbon in the cells has not been demonstrated in the diatoms, and as AZA did not significantly stimulate photorespiration in *N. ruttneri*, it seems likely that some component(s) other than carbonic anhydrase plays a more important role in suppressing photorespiration in the diatoms.

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References

Aizawa, K. and Miyachi, S. 1984. Carbonic anhydrase located on the cell surface increased the affinity for inorganic carbon in photosynthesis of *Dunaliella tertiolecta*. FEBS Lett. **173:** 41–44.

Badger, M. R., Kaplan, A. and Berry, J. A. 1980. Internal inorganic carbon pool of *Chlamydomonas reinhardtii*. Evidence for carbon dioxide-concentrating mechanism. Plant Physiol. **66:** 407–413.

Darley, W. M. and Volcani, B. E. 1971. Synchronized Cultures: Diatoms. Methods Enzymol. **23:** 85–96.

Dixon, G. K. and Merrett, M. J. 1988. Bicarbonate utilization by the marine diatom *Phaeodactylum tricornutum* Bohlin. New Phytol. **109:** 47–51.

Imamura, M., Tsuzuki, M., Shiraiwa, Y. and Miyachi, S. 1983. Form of inorganic carbon utilized for photosynthesis in *Chlamydomonas reinhardtii*. Plant Cell Physiol. **24:** 533–540.

Kimpel, D. L., Togasaki, R. K. and Miyachi, S. 1983. Carbonic anhydrase in *Chlamydomonas reinhardtii* I. Localization. Plant Cell Physiol. **24:** 255–259.

Lloyd, N. D. H., Canvin, D. T. and Culver, D. A. 1977. Photosynthesis and photorespiration in algae. Plant Physiol. **59:** 936–940.

Marcus, Y., Volokita, M. and Kaplan, A. 1984. The location of the transporting system for inorganic carbon and the nature of the form translocated in *Chlamydomonas reinhardtii*. J. Exp. Bot. **35:** 1136–1144.

Miyachi, S. and Okabe, K. 1976. Oxygen enhancement of photosynthesis in *Anacystis nidulans* cells. Plant Cell Physiol. **17:** 973–986.

Moroney, J. V. and Tolbert, N. E. 1985. Inorganic carbon uptake by *Chlamydomonas reinhardtii*. Plant Physiol. **77**: 253–258.

Moroney, J. V., Husic, H. D. and Tolbert, N. E. 1985. Effect of carbonic anhydrase inhibitors on inorganic carbon accumulation by *Chlamydomonas reinhardtii*. Plant Physiol. **79**: 177–183.

Patel, B. N. and Merrett, M. J. 1986. Inorganic carbon uptake by the marine diatom *Phaeodactylum tricornutum*. Planta **169**: 222–227.

Paul, J. S. and Volcani, B. E. 1974. Photorespiration in diatoms. I. The oxidation of glycolic acid in *Thallassiosira pseudonana* (*Cyclotella nana*). Arch. Microbiol. **101**: 115–120.

Shelp, B. J. and Canvin, D. T. 1980. Photorespiration and oxygen inhibition of photosynthesis in *Chlorella pyrenoidosa*. Plant Physiol. **65**: 780–784.

Shelp, B. J. and Canvin, D. T. 1981. Photorespiration in air and high CO_2-grown *Chlorella pyrenoidosa*. Plant Physiol. **68**: 1500–1503.

Spalding, M. H. and Ogren, W. L. 1982. Photosynthesis is required for induction of the CO_2-concentrating system in *Chlamydomonas*. FEBS lett. **145**: 41–44.

Spalding, M. H., Spreitzer, R. J. and Ogren, W. L. 1983a. Carbonic anhydrase-deficient mutant of *Chlamydomonas reinhardii* requires elevated carbon dioxide concentration for photoautotrophic growth. Plant Physiol. **73**: 268–272.

Spalding, M. H., Spreitzer, R. J. and Ogren, W. L. 1983b. Reduced inorganic carbon transport in a CO_2-requiring mutant of *Chlamydomonas reinhardii*. Plant Physiol. **73**: 273–276.

Suzuki, K. and Ikawa, T. 1984a. Effect of oxygen on photosynthetic $^{14}CO_2$ fixation in *Chroomonas* sp. (Cryptophyta) I. Some characteristics of the oxygen effect. Plant Cell Physiol. **25**: 367–375.

Suzuki, K. and Ikawa, T. 1984b. Effect of oxygen on photosynthetic $^{14}CO_2$ fixation in *Chroomonas* sp. (Cryptophyta) II. Effect of inhibitors, uncouplers and an artificial electron mediator on the inhibition of $^{14}CO_2$ fixation by anaerobiosis. Plant Cell Physiol. **25**: 377–384.

Suzuki, K. and Ikawa, T. 1985. Effect of oxygen on photosynthetic $^{14}CO_2$ fixation in *Chroomonas* sp. (Cryptophyta) III. Effect of oxygen on photosynthetic carbon metabolism. Plant Cell Physiol. **26**: 1003–1010.

Suzuki, K., Iwamoto, K., Yokoyama, S. and Ikawa, T. 1991. Glycolate oxidizing enzymes in algae. J. Phycol. **27**: 492–498.

Suzuki, K., Marek, L. F. and Spalding, M. H. 1990. A photorespiratory mutant of *Chlamydomonas reinhardtii*. Plant Physiol. **93**: 231–237.

Suzuki, K. and Spalding, M. H. 1989a. Effect of O_2 and CO_2 on CO_2 exchange in a high-CO_2-requiring mutants of *Chlamydomonas reinhardtii* during dark-light-dark transitions. Photosynth. Res. **21**: 181–186.

Suzuki, K. and Spalding, M. H. 1989b. Adaptation of *Chlamydomonas reinhardtii* high-CO_2-requiring mutants to limiting-CO_2. Plant Physiol. **90**: 1195–1200.

Sültemeyer, D. F., Miller, A. G., Espie, G. S., Fock, H. P. and Canvin, D. T. 1989. Active CO_2 transport by the green alga *Chlamydomonas reinhardtii*. Plant Physiol. **89**: 1213–1219.

Tsuzuki, M. 1983. Mode of HCO_3^- utilization by the cells of *Chlamydomonas reinhardtii* grown under ordinary air. Z. Pflanzenphysiol. **110**: 29–37.

鈴木健策・猪川倫好：淡水産ケイソウ *Nitzschia ruttneri* における光合成 $^{14}CO_2$ 固定の酸素による促進

淡水産ケイソウ *Nitzschia ruttneri* の光合成 $^{14}CO_2$ 固定は酸素により促進された。21% O_2 気相下の固定速度は0%の時と比べ15～40%高く，100% O_2 気相下でも0%の時とほぼ同じかむしろ高かった。^{14}C 固定産物では β-1,3-glucan のみが酸素に対して同様の応答を示した。固定速度に及ぼす酸素の影響は CO_2 濃度の影響を受けなかった。これは carbonic anhydrase (CA) 阻害剤 acetazolamide (AZA) 存在下でも同様であった。AZA は CO_2 に対する光合成の親和性を低下させた。非常に高い CA 活性が大気適応細胞の細胞表層に観察された。大気条件では ^{14}C のグリコール酸への取込みはほとんどみられず O_2 濃度を100%に高めても極めて低いなど，光呼吸関連物質への取込みは低く抑えられていた。本藻における「CO_2 濃縮機構」の存在が推定されたが，それと酸素促進効果との関連性は認められず，本藻の光合成の酸素促進効果はむしろ電子伝達系に関係している可能性が高い。
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