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Photorespiration and CO_2 fixation in the red alga *Porphyra yezoensis* Ueda

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Gas exchange in photosynthesis of the red alga *Porphyra yezoensis* was investigated to understand its growth enhancement in high CO_2 concentration. It was found that oxygen ($^{18}O_2$) uptake by the alga increased linearly with increased photosynthetically active radiation, being about 40% of photosynthetic oxygen evolution at 250 μmol photons $m^{-1} s^{-1}$. A substantive evidence for active HCO_3^- transport by the alga was obtained in that CO_2 uptake rate was extremely slow compared to the photosynthetic oxygen evolution rate. In the culture of the alga using aeration, dissolved inorganic carbon (DIC) concentration decreased while pH increased from the beginning of light period and increased while pH decreased from the start of dark period. This is because photosynthetic CO_2 fixation proceeded faster than the dissolution of CO_2 and thus DIC was reduced due to photosynthetic HCO_3^- utilization in the light, and in the dark the cease of photosynthesis and continuation of aeration increased DIC and lowered pH. Addition of CO_2 (air + 1250 ppm CO_2) in the culture of the alga was confirmed to raise DIC concentration. It was suggested that addition of CO_2 could suppress the photorespiration of *P. yezoensis* by elevating CO_2/O_2 ratio in the culture medium and then at the active site of Rubisco, the oxygenation of which could be subsequently reduced.

Key Index Words: *CO_2—Mehler reaction—mitochondrial respiration—O_2—photosynthesis*—Porphyra yezoensis—*red alga—Rubisco*.

The authors reported in the previous paper (Gao *et al.* 1991) that the growth of *Porphyra yezoensis* Ueda was enhanced in the culture using aeration with high CO_2. It is of general concern to understand how high CO_2 affects the efficiency of photosynthetic carbon fixation of the alga. O_2 consumption during photosynthetic O_2 evolution reflects photorespiration, which is primarily determined by the competition between CO_2 and O_2 for the active site of ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase (Rubisco). If photorespiration is considerably active in the alga, high CO_2 supply can be expected to suppress the photorespiration by raising the ratio of CO_2 to O_2 within the cell and increase the photosynthetic efficiency.

In this paper, the authors present basic knowledge on the O_2 uptake and "CO_2" transport of *P. yezoensis* while illuminated and discuss its relevance to the biological response of the alga to high CO_2 in culture.

Material and Methods

Cultures of leafy thalli of *Porphyra yezoensis* (ZW-22, ZGRW) were initiated from conchospores released from free-living conchocelis as previously reported (Gao *et al.* 1991). Thalli of 2-3 cm long (about 40 days after conchospore attachment) cultured using aeration (350 ppm CO_2) were used for the O_2 uptake experiments. The culture medium was prepared and CO_2 was supplied as in the previous paper (Gao *et al.* 1991).

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The evolution of $^{16}O_2$ and uptake of $^{18}O_2$ during photosynthesis were determined simultaneously at 10 second intervals by using a gas-permeable membrane-mass spectrometer system. A silicon membrane (125 μm thick) probe was inserted into a cylindrical reaction vessel (35 m*l*, 3 cm in diameter) to separate the medium, filtered (Whatman, GF/C) seawater, from the high-vacuum inlet to a quadrupole mass spectrometer (Ametek M200). The seawater was bubbled with N_2 for 30 minutes to expel $^{16}O_2$ before dissolving $^{18}O_2$ into it. Since the O_2 uptake reaction does not discriminate between the isotopes, correction was made for the amount of $^{16}O_2$ consumed according to Radmer and Ollinger (1980).

The reaction vessel was equipped with a water jacket and the temperature within it was controlled at 15°C.

The dissolved inorganic carbon (DIC) in the culture medium was measured by using a Shimadzu total organic analysis unit (TOC-5000).

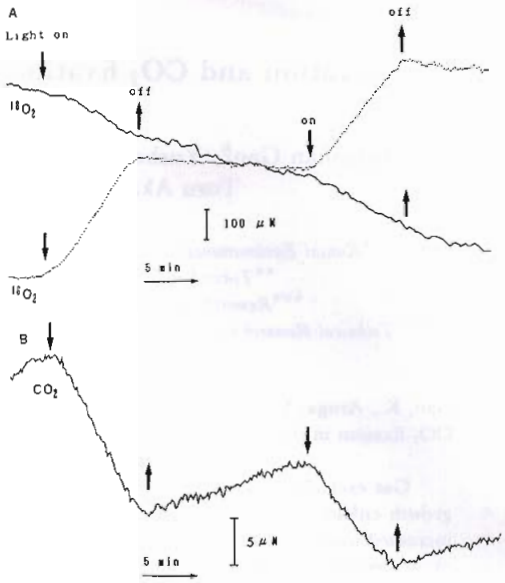

Fig. 1. Oxygen uptake and evolution (A), and CO_2 uptake (B) in photosynthesis of *Porphyra yezoensis* (ZW-22).

Results

When the light was turned on, $^{16}O_2$ increased and CO_2 decreased due to photosynthesis, whereas $^{18}O_2$ decreased due to oxygen uptake reactions; when the light was turned off, oxygen ($^{16}O_2$ and $^{18}O_2$) decreased and CO_2 increased due to mitochondrial respiration (Fig. 1). It is easy to see from the slope of $^{18}O_2$ decrease that O_2 uptake by the alga was faster in the light than in the dark (Table 1), in the former being about twice as fast as in the latter. O_2 uptake was 41 and 46% of O_2 evolution at the first and second illumination, respectively (Table 1).

The apparent CO_2 uptake rate derived from CO_2 decrease (Fig. 1B) was extraordinarily slow compared with the rate of photosynthetic O_2 evolution (Table 1). To estimate the real CO_2 uptake rate, the CO_2 supply rate from the dehydration of HCO_3^- was integrated with the apparent CO_2 uptake rate. Nevertheless, the rate of photosynthetic O_2 evolution exceeded about 13-fold the estimated real CO_2 uptake rate (Table 1). This shows a substantive evidence for HCO_3^- uptake in *P. yezoensis*. The rate of O_2 uptake in the dark exceeded the apparent rate of CO_2

Table 1. Gas exchange rate (μmol min^{-1} dm^{-2}) during photosynthesis in *Porphyra yezoensis*, the ratio of O_2 evolution to CO_2 uptake (b/c) in the light and the ratio of CO_2 to O_2 (CO_2/O_2) in the reaction vessel when light (PAR, 250 μmol photons m^{-2} s^{-1}) was turned on.

Light	O_2 upt.* (a)	O_2 evol.* (b)	Ratio a/b	CO_2 'upt.'*	CO_2 upt. (c)	Ratio b/c	CO_2 rel.*	CO_2/O_2
on	2.15	5.25	0.41	0.22 (0.19)**	0.41	12.80		0.09
off	1.10						0.04	
on	1.95	4.23	0.46	0.13 (0.19)**	0.32	13.22		0.06
off	0.97						0.03	

* upt. is for uptake, 'upt.' for apparent uptake, evol. for evolution, rel. for release.
** Values in parentheses are CO_2 supply rate (μmol CO_2 min^{-1}) from dehydration of HCO_3^- at 15°C (Cook *et al.* 1986) within the reaction vessel.

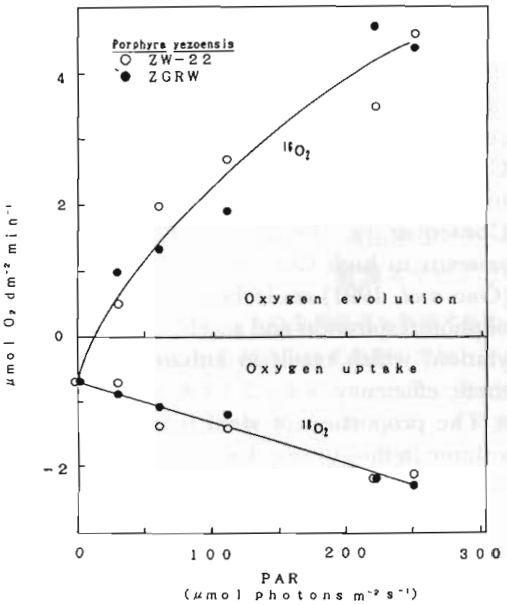

Fig. 2. Oxygen uptake and evolution in *Porphyra yezoensis* as a function of photosynthetically active radiation (PAR).

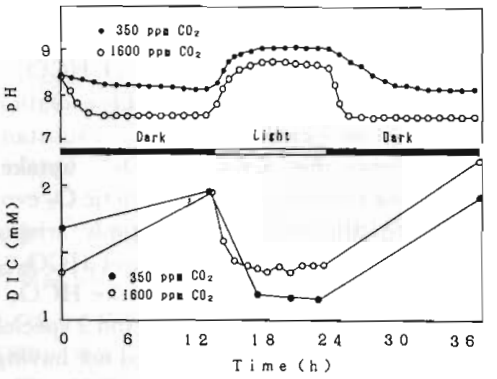

Fig. 3. Daily variation of pH and DIC (dissolved inorganic carbon) in culture of *Porphyra yezoensis* (ZGRW) using aeration (0.5 *l* min^{-1}) with air (350 ppm CO_2) and air + 1250 (1600) ppm CO_2. About 1.25 g (fresh weight) of thalli (38 days after concospore attachment, about 3 cm long) were maintained in 500 m*l* culture medium in a culture flask.

release 28- to 32-fold, which might be due to hydration of CO_2 to form HCO_3^- inside the algal cell (Table 1).

The oxygen uptake increased linearly and the oxygen evolution increased parabolically with the increase of PAR (photosynthetically active radiation, 400–700 nm) (Fig. 2). O_2 uptake of *P. yezoensis* while illuminated was similarly observed in the strains, ZW-22 and ZGRW.

Figure 3 shows daily variations of DIC and pH in the culture of *P. yezoensis* aerated with air (control) and air + 1250 ppm CO_2. DIC decreased in the light period and increased in the dark period to reach a constant level. The pH variation shows an opposite pattern to DIC. DIC was elevated and pH was lowered in + 1250 ppm CO_2 culture compared with the control.

Discussion

In the present study, basic knowledge was obtained on the gas exchange during the photosynthesis of *P. yezoensis*. O_2 uptake was enhanced in the light and increased with increase of PAR. Oxygen uptake in the light stems from the following three reactions: 1) mitochondrial respiration, 2) Mehler reaction and 3) RuBP (ribulose-1,5-bisphosphate) oxygenation. Mitochondrial respiration is independent of light, but Mehler reaction and RuBP oxygenation are light-dependant. The enhanced O_2 uptake of *P. yezoensis* in the light is due to reactions 2) and 3). The proportion of O_2 uptake to evolution was higher at low PAR than at high PAR (Fig. 2), which implies that increment in O_2 uptake with increasing PAR is slower than that in photosynthetic O_2 evolution. This must be due to much more active RuBP oxygenation at dim light when photosynthesis was much more light-limited. RuBP oxygenation is an inescapable consequence of CO_2 fixation during photosynthesis (Beardall 1989). Because Rubisco catalyses both carboxylation of CO_2 and RuBP oxygenation, O_2 concentration affects RuBP oxygenation. In the present investigation, O_2 increased and CO_2 decreased with proceeding of photosynthesis in the closed reaction vessel (Fig. 1, Table 1), therefore, the ratio of CO_2/O_2 at the active site of Rubisco might be lowered to get close to that outside the algal cell (Table 1), resulting in an enhanced oxygen uptake in the second illumination.

As previously reported (Gao *et al.* 1991), the pH rise in the culture of *P. yezoensis* (Fig. 3) is due to photosynthetic use of HCO_3^-. The high ratio of photosynthetic O_2 evolution to CO_2 uptake (Table 1) provides a substantive evidence for active HCO_3^- uptake. Comparing the rate of photosynthetic O_2 evolution with the rate of CO_2 supply arising from non-catalyzed dehydration of HCO_3^-, Cook *et al.* (1986) demonstrated the HCO_3^- uptake in 15 species of red algae and 2 species of brown algae which were proved not having extracellular carbonic anhydrase (CA). The rate of photosynthetic O_2 evolution in *Porphyra occidentalis* was reported to exceed 15.9-fold the CO_2 supply rate (Cook *et al.* 1986). In the present study, the rate of photosynthetic O_2 evolution exceeded about 13-fold the rate of CO_2 uptake in *P. yezoensis*, which probably does not possess extracellular CA. In the dark, on the other hand, the rate of O_2 uptake was about 30 times higher than the rate of CO_2 release (Table 1). This shows that CO_2 arising from mitochondrial respiration could be hydrated with the catalysis of intracellular CA to form HCO_3^-, supplementing the intracellular pool of inorganic carbon which had been depleted in the light, so that only a small amount of CO_2 was released into the medium (Table 1).

In the culture of *Porphyra yezoensis* aerated with air and air $+1250$ ppm CO_2, constant levels of pH and DIC in the middle of light period (Fig. 3) indicate the balance between the photosynthetic carbon fixation and dissolution of CO_2 from air into the culture medium. DIC level was reduced at about 1.5 mM in the middle of the light period even in the CO_2-enriched culture, where the photosynthesis of the alga must have been carbon-limited and photorespiration could be still active. However, since O_2 concentration in the culture medium should be constant because of the continuous aeration, the ratio of CO_2 to O_2 must be raised in the culture medium with addition of CO_2 and consequently at the active site of Rubisco, therefore the active photorespiration could be suppressed. The K_m (CO_2) of Rubisco from marine macroalgae is reported to be in a 30–60 μM range (Kerby and Raven 1985), which is much higher than the concentration of free CO_2 in seawater (about 14 μM at 15°C, pH 8.2). When *P. yezoensis* is cultured using aeration with high CO_2, increased partial pressure of CO_2 serves to saturate Rubisco-catalyzed carboxylation. Consequently, the enhanced growth of *P. yezoensis* in high CO_2 as reported previously (Gao *et al.* 1991) could be due to suppression of photorespiration and acceleration of carboxylation, which result in enhanced photosynthetic efficiency.

The proportion of algal biomass to water volume in the culture (Fig. 3) was 2.5 kg m^{-3}. Growth of the alga was shown to be enhanced in air $+650$ and $+1250$ CO_2 cultures with the proportion of biomass to water volume being less than 0.5 kg m^{-3} (Gao *et al.* 1991). It is suggested that growth of the alga in cultivation site might be CO_2-limited when seawater exchange is slow between the inside and the outside of the algal population. Since increase in seawater current speed increases the photosynthesis of *P. yezoensis* (Gao *et al.* 1992), fast seawater flow through the algal population can be expected to lessen photorespiration and give rise to less CO_2-limited growth by taking away photosynthesis-evolved O_2 and resupplying 'CO_2'.

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高　坤山*・有賀祐勝**・浅田浩二***・石原利章****・赤野　徹****・清原正高****：

紅藻スサビノリの光呼吸と CO_2 固定

高 CO_2 によるスサビノリの生長促進を解明するためにその光合成時のガス交換を調べた。スサビノリの光照射下での酸素 ($^{18}O_2$) の取り込みは光強度の増加とともに直線的に増加し，250 μmol photons m^{-2} s^{-1} において酸素発生の約40%に達することを明らかにした。光合成時に CO_2 の取り込み速度が酸素発生速度より著しく下回ったことはスサビノリの HCO_3^- を取り込むことを裏付ける。一方，通気培養の場合，明期にはpHの上昇に伴い溶存無機炭素 (DIC) 濃度が低下し，暗期にはpHの低下に伴いDIC濃度が増加することが分かった。これは明期には光合成による CO_2 固定が空気から培養液への CO_2 の溶け込みを上回るため培養液中の HCO_3^- が利用され，暗期には通気による CO_2 のとけ込みだけが進んだ結果である。また，CO_2 の添加（空気＋1250 ppm）によって培養液中の無機炭素濃度が高められたことが確認された。スサビノリの通気培養では，CO_2 の添加によって高まった培養液での CO_2/O_2 が細胞内の CO_2/O_2 を高めてRubiscoのoxygenaseとしての活性を抑えることによってその光呼吸が抑えられるものと推測された。（*530 大阪市北区中崎西2-3-39　関西総合環境センター (present address: Hawaii Natural Energy Institute, 2540 Dole Street, Holmes 246, Honolulu, HI 96822, U.S.A.), **108 東京都港区港南4-5-7　東京水産大学，***611 京都府宇治市　京都大学食糧科学研究所，****661 兵庫県尼崎市若王子3-11-20　関西電力総合技術研究所）