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The Effect of Elevated Partial Pressures of CO_2 on the Relationship between Photosynthetic Capacity and N Content in Rice Leaves[1]

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The effects of growth CO_2 levels on the photosynthetic rates; the amounts of ribulose-1,5-bisphosphate carboxylase (Rubisco), chlorophyll (Chl), and cytochrome *f*; sucrose phosphate synthase activity; and total N content were examined in young, fully expanded leaves of rice (*Oryza sativa* L.). The plants were grown hydroponically under two CO_2 partial pressures of 36 and 100 Pa at three N concentrations. The light-saturated photosynthesis at 36 Pa CO_2 was lower in the plants grown in 100 Pa CO_2 than those grown in 36 Pa CO_2. Similarly, the amounts of Rubisco, Chl, and total N were decreased in the leaves of the plants grown in 100 Pa CO_2. However, regression analysis showed no differences between the two CO_2 treatments in the relationship between photosynthesis and total N or in the relationship between Rubisco and Chl and total N. Although a relative decrease in Rubisco to cytochrome *f* or sucrose phosphate synthase was found in the plants grown in 100 Pa CO_2, this was the result of a decrease in total N content by CO_2 enrichment. The activation state of Rubisco was also unaffected by growth CO_2 levels. Thus, decreases in the photosynthetic capacity of the plants grown in 100 Pa CO_2 could be simply accounted for by a decrease in the absolute amount of leaf N.

Leaf photosynthesis is affected by the levels of environmental CO_2 in which the plants are grown. Short-term (seconds to hours) CO_2 enrichment stimulates the photosynthetic rate per unit of leaf area. Plant mass is also enhanced during a subsequent long-term exposure (weeks to months) to elevated CO_2 levels. However, such a long-term CO_2 enrichment reduces the initial stimulation of photosynthesis and then frequently suppresses photosynthesis (for reviews, see Stitt, 1991; Bowes, 1993; Sage, 1994). These findings indicate that prolonged exposure to elevated CO_2 leads to changes in biochemical, physiological, or morphological factors, which remove or offset the initial stimulation of photosynthesis. However, the mechanisms of the suppression of photosynthesis by long-term CO_2 enrichment still remain unclear.

Growth under high CO_2 partial pressures leads to carbohydrate accumulation. Since an apparent correlation between carbohydrate accumulation and the suppression of photosynthesis has often been reported (Sasek et al., 1985; Peet et al., 1986; Yelle et al., 1989; Wong, 1990; Xu et al., 1994), much attention has been paid to the causal relationship(s) between the two phenomena. Stitt (1991) proposed the existence of a feedback mechanism(s) by which the accumulation of carbohydrate indirectly leads to a decrease in the amounts of key components of the photosynthetic apparatus. In fact, many studies of long-term acclimation to elevated CO_2 partial pressures have shown a decrease in Rubisco (Peet et al., 1986; Sage et al., 1989; Rowland-Bamford et al., 1991; Xu et al., 1994; Jacob et al., 1995; Rogers et al., 1996). In addition, a decrease in the transcript levels of *rbc*S and *rbc*L mRNA was recently found in the plants grown at elevated CO_2 partial pressures (Nie et al., 1995; van Oosten and Besford, 1995). However, although there has been some evidence of the regulation of the expression of several photosynthetic genes by an increased soluble hexose concentration (Sheen, 1990; Krapp et al., 1993; van Oosten and Besford, 1996), it is still unknown whether there is a direct causal relationship between carbohydrate accumulation and the suppression of photosynthesis.

The suppression of photosynthesis by CO_2 enrichment is also associated with a decrease in total leaf N content (Rowland-Bamford et al., 1991; Conroy and Hocking, 1993; Tissue et al., 1993; Delgado et al., 1994; Koike et al., 1995; Rogers et al., 1996; Roumet et al., 1996). Since photosynthesis is determined by the absolute N content and N partitioning in a leaf, the decreases in Rubisco and other key components of photosynthesis by CO_2 enrichment must be evaluated in relation to reduced leaf N content. Under the conditions of CO_2 enrichment photosynthesis is limited by either electron-transport capacity or Pi-regeneration capacity during starch and Suc synthesis and is not limited by Rubisco (Farquhar et al., 1980; Sharkey, 1985). However, it is unclear whether plants are potentially able to acclimatize to elevated CO_2 and reduce Rubisco to the optimal levels under the conditions of CO_2 enrichment (Medlyn, 1996). Makino (1994) reported that growth CO_2

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Abbreviations: Chl, chlorophyll; *pCa*, ambient CO_2 partial pressure; *pCi*, intercellular CO_2 partial pressure; SPS, Suc phosphate synthase.

levels did not affect Rubisco content and electron-transport activity for a given leaf N content. Similar results were found by Thomas et al. (1994). However, there have been a few reports showing that plants grown under CO_2 enrichment contain a decreased amount of Rubisco relative to leaf N content (Sage et al., 1989; Rowland-Bamford et al., 1991; Rogers et al., 1996). Thus, it also remains uncertain whether the decrease in leaf N content by CO_2 enrichment is associated with changes in N partitioning among key components of photosynthesis, including Rubisco.

In this study we grew rice (*Oryza sativa* L.) plants hydroponically under two CO_2 partial pressures of 36 and 100 Pa, at three N concentrations, and investigated how the growth CO_2 levels affected the photosynthetic rate, absolute N content, and N partitioning in single leaves. We measured Rubisco as a determinant for CO_2-limited photosynthesis (von Caemmerer and Farquhar, 1981), Chl as a light-harvesting component, Cyt *f* as a rate-limiting factor for electron transport (Price et al., 1995), and SPS as a key enzyme during Suc synthesis (Huber and Huber, 1996). In addition, we measured the gas-exchange rates as a function of *pCi* to deduce the in vivo balance among those processes limiting photosynthesis. Finally, to evaluate the changes in photosynthesis and these key components by CO_2 enrichment in relation to leaf N content, we analyzed their relationships to leaf N content and characterized the photosynthetic system acclimated to elevated partial pressures of CO_2.

MATERIALS AND METHODS

Plant Culture

Rice (*Oryza sativa* L. cv Notohikari) plants were grown hydroponically in an air-conditioned greenhouse with a day/night temperature of 25/20°C under natural sunlight conditions. After germination the seedlings were grown on a plastic net floating on tap water adjusted to pH 5.5 for 3 weeks. Four seedlings each were then transplanted to a 3.5-L plastic pot containing nutrient solution. The basal nutrient solution was as previously described by Makino et al. (1988). The solution was renewed once a week. When the eighth leaf blades emerged, plants were transferred to an environmentally controlled growth chamber equipped with a CO_2 partial pressure regulator (Eyelatron model FLI-301NHCL, Eyela, Tokyo). The chamber was operated with a 15-h photoperiod, 25/20°C day/night temperature, 60% RH, and a PPFD of 850 μmol quanta m^{-2} s^{-1} at plant level during the daytime. Irradiance was provided by a combination of a metal halide lamp (Yoko DR, Toshiba, Tokyo), high-output fluorescent lamps (FL40SS-EX, Toshiba/Panasonic, Tokyo), and True-Lite lamps (Doro-Test, North Bergen, NJ). After 1 week the plants were grown under two CO_2 partial pressures, 36 ± 4 and 100 ± 5 Pa. For each CO_2 treatment, N concentrations (mM) in the hydroponic solutions were 0.5 (0.25 mM NH_4NO_3), 2.0 (1.0 mM NH_4NO_3), and 8.0 (2.5 mM NH_4NO_3 plus 3.0 mM $NaNO_3$). These solutions were renewed once a week and continuously aerated. Gas exchange and biochemical assays were carried out on young, fully expanded leaves of the plants grown under different CO_2 partial pressures for about 3 weeks.

Gas-Exchange Measurements

Gas exchange was determined with an open gas-exchange system (Makino et al., 1988). Differences in the partial pressures of CO_2 and H_2O entering and exiting the chamber were measured with an IR gas analyzer (ASSA-1110, Horiba, Tokyo) and a dew-point hygrometer (model 911, EG&G, Natick, MA), respectively. Measurements were made at a PPFD of 1700 μmol quanta m^{-2} s^{-1}, a leaf temperature of 25°C, and a leaf-to-air vapor difference of 1.0 to 1.2 kPa. The first measurement was made at a *pCa* of 36 Pa until the steady state of the gas-exchange rate was obtained, and then *pCa* was varied to measure the rates at a *pCi* of 20 Pa and greater than 60 Pa. Gas-exchange parameters were calculated according to the equations of von Caemmerer and Farquhar (1981).

Biochemical Assays

The amounts of Chl, total leaf N, Rubisco, and Cyt *f* were determined according to the methods of Makino et al. (1994a). The leaf blade that had been used for the gas-exchange measurements was homogenized in 50 mM sodium phosphate buffer (pH 7.0) containing 120 mM 2-mercaptoethanol, 2 mM iodoacetic acid, and 5% (v/v) glycerol. The total Chl and leaf N contents were measured from part of this homogenate. To solubilize membrane-bound Rubisco, Triton X-100 to a final concentration of 0.1% (v/v) was added to a portion of the leaf homogenate (Makino and Osmond, 1991). After centrifugation, the amount of Rubisco in the supernatant fluid was measured spectrophotometrically after formamide extraction of Coomassie brilliant blue R-250-stained subunit bands separated by SDS-PAGE. A calibration curve was obtained with Rubisco purified from rice leaves. The relative amount of Cyt *f* was determined by rocket immunoelectrophoresis after solubilization of the precipitate fraction with lithium dodecyl sulfate, according to the method of Plumley and Schmidt (1983), with a slight modification (Makino et al., 1994a). The specific antibody against Cyt *f* used was previously prepared (Hidema et al., 1991).

Rubisco activation was determined according to the method of Makino et al. (1994b). When the steady-state rate of photosynthesis was attained with the gas-exchange system, the leaf was rapidly frozen in liquid N_2 and immediately used for Rubisco assay. The activation state of Rubisco was calculated as the initial activity of Rubisco in extracts assayed at 25°C within 120 s from the start of extraction, divided by the activity in extracts incubated at 25°C for 7 min with 20 mM $MgCl_2$ and 20 mM $NaHCO_3$ (pH 8.2). The Rubisco activity was carried out at 25°C for 1 min in 100 mM Bicine-NaOH (pH 8.2) containing 25 mM $MgCl_2$, 5 mM $NaH^{14}CO_3$ (18 MBq $mmol^{-1}$), and 0.6 mM ribulose-1,5-bisphosphate.

SPS activity was measured on a subsample of each treatment group by the method of Huber et al. (1989), as de-

scribed by Nakano et al. (1995). The assay was carried out at 25°C under V_{max} substrate conditions.

Suc and Starch Analysis

For a determination of Suc and starch the leaf blades were collected just before nighttime and dried at 80°C. Dry materials were milled and extracted at 80°C with 80% (v/v) ethanol. The Suc content was determined by the method of Jones et al. (1977), as described by Nakano et al. (1995). Starch in the ethanol-insoluble fraction was extracted with 0.5 M KOH and neutralized with 0.5 M $HClO_4$. After removal of $KClO_4$ was removed, the starch was digested with amyloglucosidase (Nakano et al., 1995). The Glc content was determined by the method of Somogyi (1952), and the amount of starch was calculated by multiplying its Glc content by 0.9.

RESULTS

Light-saturated photosynthesis measured at a *pCa* of 36 Pa was appreciably lower in the elevated-CO_2-grown (100 Pa) plants than in the normal-CO_2-grown (36 Pa) plants for all N treatments (Fig. 1). This suggests an apparent suppression of photosynthesis by CO_2 enrichment, but this effect of growth CO_2 depended on the N nutrition status. For example, the ratio of decreased photosynthesis in the elevated-CO_2-grown plants was 36% for the 0.5 mM N treatment, whereas it was 18% for the 8 mM N treatment. Similarly, Rubisco and Chl contents were decreased in the leaves of the plants grown at elevated CO_2 partial pressures (Fig. 2), and their decreases were also larger in the plants grown in low N concentrations than in the plants grown in high N concentrations. In addition, the responses of Rubisco and Chl contents to growth CO_2 and N nutrition were correlated with that of total leaf N content. Thus, growth under elevated CO_2 partial pressures led to a decrease in the photosynthetic capacity, and this decrease is suggested to be closely related to a decline in leaf N content. The accumulation of carbohydrates was also found in the leaf blades of the plants grown under elevated CO_2 partial pressures (Fig. 3). Although rice belongs to a species that preferentially accumulates Suc as the carbohydrate (Nakano et al., 1995), a great deal of starch accumulation was found in the plants grown under elevated CO_2 partial pressures in the present study. However, starch content decreased with increasing N supply, whereas Suc content increased. The decrease in photosynthesis by CO_2 enrichment was correlated with the starch accumulation.

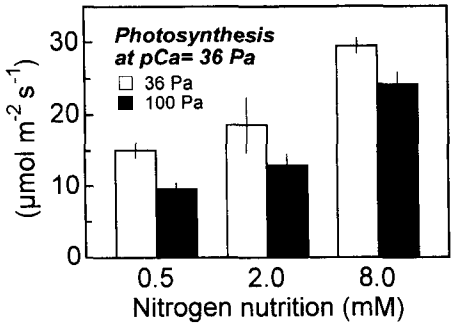

Figure 1. Rate of photosynthesis at a *pCa* of 36 Pa in leaves of rice grown hydroponically under two CO_2 partial pressures of 36 and 100 Pa CO_2 and N concentrations of 0.5, 2.0, and 8.0 mM. Measurements were made at a PPFD of 1700 μmol quanta m^{-2} s^{-1}, a leaf temperature of 25°C, and a leaf-to-air vapor pressure difference of 1.0 to 1.2 kPa. The vertical bar on each column indicates the SE ($P < 0.05$, n = 4–10).

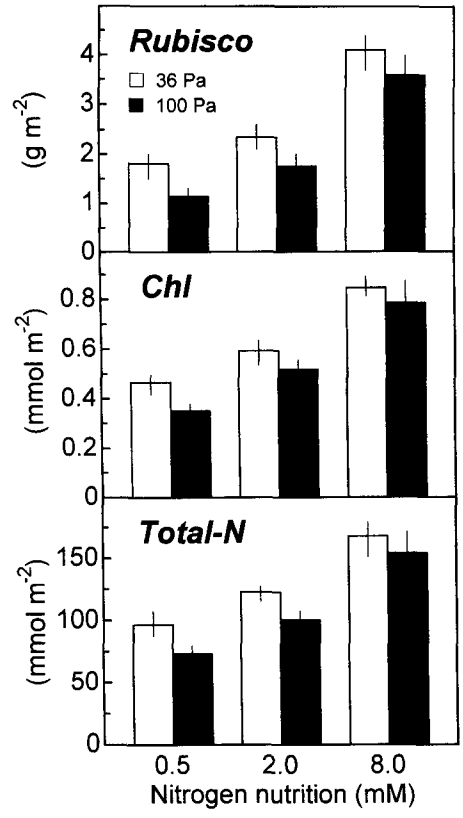

Figure 2. Rubisco, Chl, and total N contents in leaves of rice. Column symbols are the same as in Figure 1. The vertical bar on each column indicates the SE ($P < 0.05$, n = 4–5).

Table I shows the effect of growth CO_2 on the ratio of Cyt *f* content to Rubisco content and the ratio of SPS activity to Rubisco content. For each N treatment, these ratios were greater in the plants grown in high CO_2 partial pressures than in those grown in low CO_2 partial pressures. Since photosynthesis at elevated CO_2 is limited by either electron-transport capacity or Pi-regeneration capacity during starch and Suc synthesis, the data in Table I suggest that N allocation from Rubisco to components limiting photosynthesis at elevated CO_2 apparently occurred in the plants grown in high CO_2 partial pressures. However, these ratios also tended to increase with decreasing N supply, irrespective of CO_2 treatment. When the relationships between Rubisco, Chl, and Cyt *f* contents and SPS activity versus total leaf N content were analyzed, no difference between the two CO_2 treatments was found for any of the components or activities at a given leaf N content (Fig. 4). These results indicate that the decrease in the key

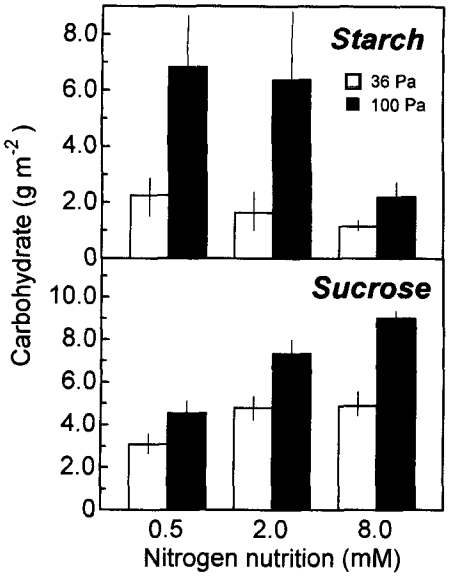

Figure 3. Starch and Suc contents in leaves of rice. Column symbols are the same as in Figure 1. The vertical bar on each column indicates the SE ($P < 0.05$, $n = 4–5$).

components of photosynthesis and the changes in the ratio among the components by CO_2 enrichment are the result of a decrease in total leaf N content and that the changes in N partitioning among photosynthetic components are essentially independent of growth CO_2. Thus, the changes in N content by growth CO_2 make it very difficult to evaluate changes in N allocation to Rubisco and other components of photosynthesis.

Gas-exchange studies can also indicate whether the in vivo ratios of electron transport and of Pi regeneration to Rubisco capacities are affected by growth CO_2 levels. According to the photosynthetic model for C_3 species developed by Farquhar et al. (1980), the photosynthetic rate at low *pCi* is limited by Rubisco, whereas the rate at high *pCi* is limited by electron-transport capacity. In addition, photosynthesis under saturating *pCi* can be limited by the capacity of Pi regeneration during starch and Suc synthesis (Sharkey, 1985). Therefore, if the in vivo ratios are affected by growth CO_2 levels, we can expect to observe a difference in the ratio of the photosynthetic rate at low *pCi* to the rate at high *pCi*. Sage (1994) reviewed more than 40 gas-exchange studies of the *pCi* response of photosynthesis and reported that the shape of the *pCi*-response curve of photosynthesis changes depending on the levels of growth CO_2.

Figure 5 shows the relationships between the photosynthetic rates at a *pCi* of 20 Pa and greater than 60 Pa and the ratio of their rates versus total leaf N content. There was no difference between CO_2 treatments in both the rates of photosynthesis at any given leaf N content, and the ratio of the rates at high *pCi* to low *pCi* did not depend on growth CO_2 but did depend on leaf N content. Thus, although a decrease in the ratio of the photosynthetic rate at high *pCi*

Table I. *Ratios of Cyt f to Rubisco contents and of SPS activity to Rubisco in leaves of rice grown under different CO_2 partial pressures and N concentrations*

Values in parentheses show the relative ratios for each N treatment at 36 Pa CO_2. No statistical analysis was done.

N Treatment	Growth CO_2	Cyt *f*/Rubisco	SPS/Rubisco
mM	*Pa*	*relative amount* g^{-1}	$\mu mol\ s^{-1}\ g^{-1}$
0.5	36	0.264 (100)	1.49 (100)
	100	0.354 (134)	2.68 (180)
2.0	36	0.279 (100)	1.67 (100)
	100	0.342 (123)	2.09 (125)
8.0	36	0.215 (100)	1.28 (100)
	100	0.231 (107)	1.45 (113)

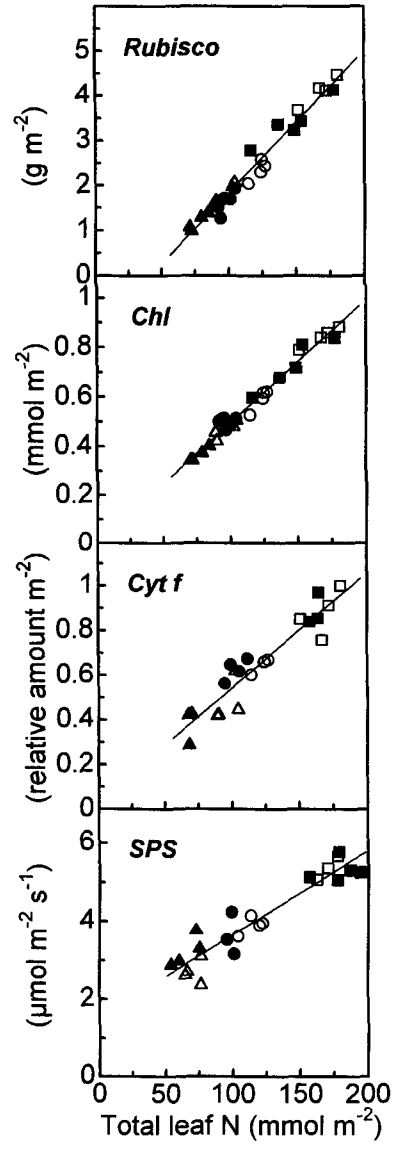

Figure 4. Rubisco content, Chl content, Cyt *f* content, and SPS activity versus total leaf N content. Plants were grown hydroponically under two CO_2 partial pressures of 36 (open symbols) and 100 (closed symbols) Pa CO_2 and N concentrations of 0.5 (triangle), 2.0 (circle), and 8.0 (square) mM. For Rubisco, $Y = 0.0327X - 1.33$, $r^2 = 0.97$; Chl, $Y = 0.00498X - 0.003$, $r^2 = 0.97$; Cyt *f*, $Y = 0.0521X + 0.023$, $r^2 = 0.89$; and SPS, $Y = 0.0215X + 1.51$, $r^2 = 0.89$.

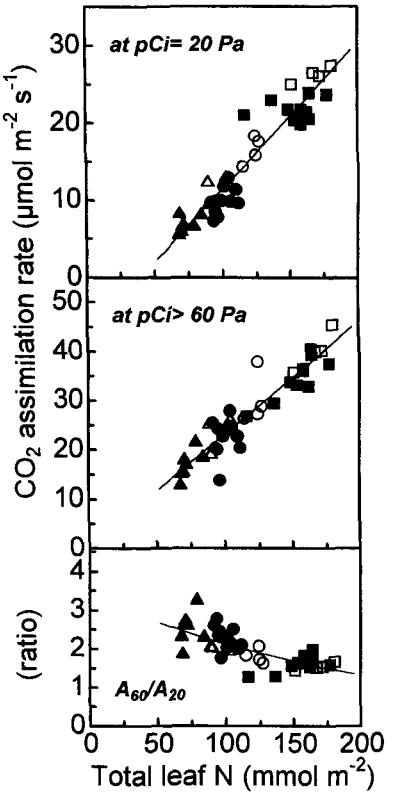

Figure 5. Rates of photosynthesis at a *pCi* of 20 Pa (A_{20}) *and pCi* of >60 Pa (A_{60}) and the ratio of the rates at a *pCi* of >60 Pa to a *pCi* of 20 Pa versus total leaf N content. Symbols are the same as in Figure 4. Photosynthesis was measured at a PPFD of 1700 μmol quanta m^{-2} s^{-1}, a leaf temperature of 25°C, and a leaf-to-air vapor pressure difference of 1.0 to 1.2 kPa. For A_{20}, $Y = 0.189X - 7.46$, $r^2 = 0.90$; *for* A_{60}, $Y = 0.231X + 0.01$, $r^2 = 0.87$.

to low *pCi* by CO_2 enrichment was found within the same N treatments, this was caused by decreased leaf N content. In addition, the suppression of photosynthesis by CO_2 enrichment could also be accounted for by the decrease in leaf N content.

Last, we examined the activation state of Rubisco at a low *pCi* and high *pCi* of each CO_2 treatment (Fig. 6). The growth CO_2 levels did not affect Rubisco activation. Although the activation at high *pCi* declined substantially with increasing N supply, this trend was found for both CO_2 treatments. The catalytic turnover rate of fully activated Rubisco was unaffected by growth CO_2 (data not shown). These results also suggest that, although the in vivo balance among the capacities of the above three processes limiting photosynthesis is affected by N nutrition, it is not affected by CO_2 enrichment.

DISCUSSION

Our results show that the decrease in the photosynthetic rate per unit of leaf area by CO_2 enrichment can be accounted for by a decrease in the absolute amount of leaf N. The accumulation of carbohydrates did not directly affect photosynthesis. Although a selective decrease in Rubisco content relative to other photosynthetic components at the elevated CO_2 level was found, this was simply caused by a decrease in leaf N content during the long-term exposure to a high partial pressure of CO_2. CO_2 enhancement also did not affect Rubisco activation. Here we discuss each of these findings in detail and describe the physiological implications of a decrease in leaf N by CO_2 enrichment.

Accumulation of Carbohydrates and Suppression of Photosynthesis

Growth at elevated CO_2 levels leads to the accumulation of carbohydrates in leaves. Therefore, much attention has been paid to whether there is evidence of feedback mechanisms in which the accumulation of carbohydrates can act directly or indirectly to inhibit photosynthesis. Starch accumulation is generally thought to physically distort the chloroplast (Cave et al., 1981; DeLucia et al., 1985) and possibly hinder CO_2 diffusion within the chloroplast (Makino, 1994). Increased soluble sugars lead to a down-regulation of the gene expression of several key components of photosynthesis (Sheen, 1990; Krapp et al., 1993; van Oosten and Besford, 1996). However, our present results showed that, in spite of the accumulation of starch and Suc (Fig. 3), these did not affect photosynthesis for a given leaf N content. Similar results were previously found in the flag leaves of panicle-removed rice plants (Nakano et al., 1995); this is probably because the absolute amounts of these carbohydrates accumulating in leaves of rice are small compared with those in other species. For example, the amount of starch in bean plants grown under the same conditions was 6-fold greater than that in rice and reached 37 g m^{-2} (data not shown). Rice plants can accumulate a

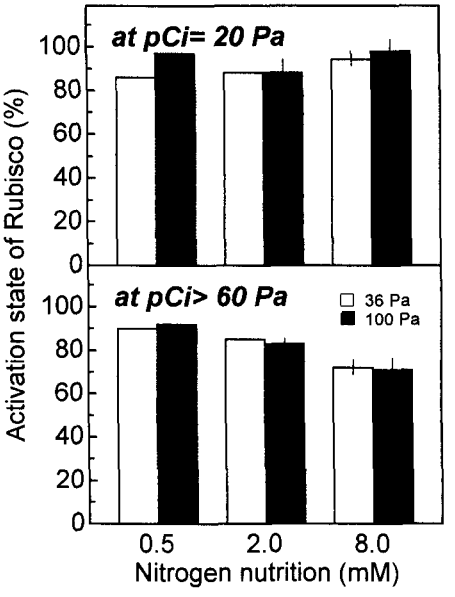

Figure 6. Activation state of Rubisco in leaves under the conditions of a PPFD of 1700 μmol quanta m^{-2} s^{-1}, a leaf temperature of 25°C, and the indicated partial pressures of CO_2. Column symbols are the same as in Figure 1. The vertical bar on each column indicates the SE ($P < 0.05$, $n = 4$).

great deal of carbohydrates in their leaf sheaths. In fact, 5- to 10-fold amounts of carbohydrates per plant part were found in the leaf sheath (data not shown). Therefore, in our experiments with rice the accumulation of carbohydrates in the leaves may have had no direct effect on photosynthesis. However, it is still possible that the accumulation of carbohydrates directly or indirectly led to an decrease in leaf N content, which resulted in decreased photosynthesis.

Changes in N Partitioning in a Leaf during Long-Term CO_2 Enrichment

Photosynthesis at elevated CO_2 levels is limited by electron-transport or Pi-regeneration capacity but not by Rubisco (Farquhar et al., 1980; Sharkey, 1985). Therefore, if plants are potentially able to optimize N allocation at elevated CO_2 levels, N from Rubisco, the most abundant leaf protein, should be reallocated into electron-transport components and/or key enzymes of starch and Suc synthesis during long-term CO_2 enrichment (Medlyn, 1996). Contrary to this expectation, however, our results showed that CO_2 enrichment did not reduce Rubisco content; nor did it enhance Cyt *f* content or SPS activity for a given leaf N content (Fig. 4). In agreement with these results, no difference due to growth CO_2 levels was found for both CO_2-limited and CO_2-saturated photosynthesis at any given leaf N content (Fig. 5). On the other hand, there have been a few reports that Rubisco content declines relative to leaf N content during long-term CO_2 enrichment when N is limiting for growth (Sage et al., 1989; Rowland-Bamford et al., 1991; Rogers et al., 1996). However, these findings remain uncertain because the decrease in N content by CO_2 enrichment makes it difficult to evaluate the change in N allocation to Rubisco and other components of photosynthesis. When N supply is low, N allocation to Rubisco is reduced relative to other components of photosynthesis (Evans and Terashima, 1988; Makino et al., 1992, 1994a). According to our present results, this phenomenon was independent of growth CO_2 levels. Indeed, in our results with rice a decrease in Rubisco relative to Cyt *f* content or relative to SPS activity was also found within the same N treatment (Table I); however, this was not the direct result of CO_2 enrichment but rather was the result of a decrease in leaf N induced by CO_2 enrichment. Thus, although long-term CO_2 enrichment leads to a decrease in Rubisco, this does not indicate an optimization of N partitioning at elevated CO_2 levels.

Rubisco Activation at Elevated CO_2

It has been frequently reported that CO_2 enhancement decreases the activation state of Rubisco in several C_3 species such as *Raphanus sativus* (von Caemmerer and Edmondson, 1986), bean (Sage et al., 1988; Socias et al., 1993), lamb's-quarters, cabbage, and eggplant (Sage et al., 1989). Sage et al. (1988) suggested that the deactivation of Rubisco is a secondary response to the maintenance of the balance between Rubisco and other processes limiting photosynthesis at elevated CO_2 levels. In addition, Sage et al. (1989) found that the deactivated Rubisco immediately after exposure to elevated CO_2 levels does not recover during the subsequent prolonged exposure, and they pointed out that this indicates an incomplete acclimation to elevated CO_2 levels. On the other hand, there have been some reports that the activation state of Rubisco at elevated CO_2 partial pressures is unchanged in soybean (Campbell et al., 1988; Sicher et al., 1995), loblolly pine (Tissue et al., 1993), tobacco (Sicher and Kremer, 1994), and pea (Xu et al., 1994). In rice Rowland-Bamford et al. (1991) observed that Rubisco activation declined in the plants grown at 90 Pa CO_2, whereas it was not affected in the plants grown at CO_2 levels up to 60 Pa. In our study of rice, Rubisco activation remained at high levels and was completely independent of growth CO_2 levels (Fig. 6). Although the activation state at a high CO_2 partial pressure declined substantially with increasing N supply, this was probably caused by a Pi-regeneration limitation during starch and Suc synthesis. As leaf N content increases in rice, this limitation strongly comes into play with high CO_2 partial pressures (Makino et al., 1994a). Thus, there seems to be a species-dependent difference in the short-term response of Rubisco activation to elevated CO_2 levels, but growth under CO_2 enrichment may have no effect on Rubisco activation. If Rubisco activation is determined by the balance among the capacities of Rubisco, electron transport, and starch and Suc synthesis (Sage, 1990), our results also indicate that the decrease in leaf photosynthesis during CO_2 enrichment is not associated with changes in the in vivo balance among these three photosynthesis-limiting processes.

Implications of a Decrease in Leaf N

A decrease in leaf N content is commonly found in many C_3 plants grown under CO_2 enrichment (Conroy and Hocking, 1993; Tissue et al., 1993; Delgado et al., 1994; Koike et al., 1995; Rogers et al., 1996; Roumet et al., 1996). Our results clearly showed that the suppression of photosynthesis and an apparently selective reduction of Rubisco content are simply due to a decrease in leaf N content by CO_2 enrichment. However, we believe that the physiological implications of the decrease in leaf N content are of crucial importance for the whole-plant growth under CO_2 enrichment. This decrease in leaf N content is not due to dilution of N caused by a relative increase in leaf area or plant biomass, because the decrease in leaf N is greater in plants grown with a low N supply, whereas the enhancement of the biomass is smaller at a low concentration of N. Further work aimed at resolving these problems will be presented in our companion paper (Makino et al., 1997).

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