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Effect of CO_2 Concentration on Carbonic Anhydrase and Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase Expression in Pea[1]

Nathalie Majeau[2] and John R. Coleman*

Department of Botany, University of Toronto, 25 Willcocks Street, Toronto, Ontario, Canada M5S 3B2

The effect of external CO_2 concentration on the expression of carbonic anhydrase (CA) and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) was examined in pea (*Pisum sativum* cv Little Marvel) leaves. Enzyme activities and their transcript levels were reduced in plants grown at 1000 μL/L CO_2 compared with plants grown in ambient air. Growth at 160 μL/L CO_2 also appeared to reduce steady-state transcript levels for *rbcS*, the gene encoding the small subunit of Rubisco, and for *ca*, the gene encoding CA; however, *rbcS* transcripts were reduced to a greater extent at this concentration. Rubisco activity was slightly lower in plants grown at 160 μL/L CO_2, and CA activity was significantly higher than that observed in air-grown plants. Transfer of plants from 1000 μL/L to air levels of CO_2 resulted in a rapid increase in both *ca* and *rbcS* transcript abundance in fully expanded leaves, followed by an increase in enzyme activity. Plants transferred from air to high-CO_2 concentrations appeared to modulate transcript abundance and enzyme activity less quickly. Foliar carbohydrate levels were also examined in plants grown continuously at high and ambient CO_2, and following changes in growth conditions that rapidly altered *ca* and *rbcS* transcript abundance and enzyme activities.

As atmospheric levels of CO_2 continue to increase, there is considerable research interest in the mechanisms by which plants respond to changes in CO_2 concentration, and, in particular, how these changes have an impact on photosynthetic processes (see reviews by Bowes, 1991; Stitt, 1991; Sage, 1994; Webber et al., 1994). Many laboratory studies using growth chamber- or glasshouse-grown plants have shown that some species experience a short-term stimulation followed by a decline in photosynthetic rate after prolonged exposure to high-CO_2 (Stitt, 1991; Webber et al., 1994). This decline in photosynthesis has been associated with a reduction in Rubisco activity, although the capacity of the plant for RuBP/Pi regeneration may also limit photosynthetic rate. Studies have shown that Rubisco protein levels are lower in high-CO_2-grown plants, particularly when additional factors such as nutrient limitation, developmental stage, or root-growth space inhibit effective carbohydrate translocation from leaves into sink tissues (Sage et al., 1989; Besford et al., 1990; Rowland-Bamford et al., 1991). These additional factors are an important consideration, since some plant species grown in nonlimiting environments at high-CO_2 concentrations do not exhibit a reduction in photosynthetic capacity (Sage, 1994). Nonetheless, the observed reduction in photosynthetic capacity when source-to-sink translocation is constrained indicates that additional regulatory steps in photosynthesis are operating.

The enzyme CA (EC 4.2.1.1), which is localized primarily in the chloroplast stroma of C_3 higher plants, is thought to play a role in photosynthesis by facilitating diffusion into and across the chloroplast, as well as by catalyzing HCO_3^- dehydration to supply CO_2 for Rubisco (Badger and Price, 1994). Therefore, it would be anticipated that this enzyme would respond to changes in ambient levels of CO_2 in a manner similar to Rubisco. Previous studies have shown that CA activities are reduced in leaves of *Cucumis sativus* (Peet et al., 1986), *Avena sativa* (Cervigni et al., 1971), *Gossypium hirsutum* (Chang, 1975), and *Phaseolus vulgaris* (Porter and Grodzinski, 1984) grown at elevated CO_2 concentrations, although the mechanisms of regulation were not identified. Recently, in contrast with earlier studies showing a CO_2-induced reduction in CA activity, it was reported that chloroplast CA mRNA abundance increased during acclimation of *Arabidopsis thaliana* to elevated CO_2 levels; however, no measurements of CA or Rubisco activity were made in that study (Raines et al., 1992). In contrast to higher plant studies, the response of CA expression during acclimation to limiting levels of C_i is well documented in eukaryotic algae (Coleman, 1991; Badger and Price, 1992; Sultemeyer et al., 1993). Extracellular levels of C_i appear to regulate transcriptional activity of genes encoding various isoforms of CA, and transfer of cells from one CO_2 level to another results in rapid modification of *ca* mRNA abundance, protein level, and activity (Bailly and Coleman, 1988; Fukuzawa et al., 1990).

In this study we examined CA and Rubisco expression in pea (*Pisum sativum*) leaves acclimated to ambient, elevated, and below-ambient levels of CO_2. In addition, we have examined the capacity of the plants to modify CA and Rubisco activity and transcript abundance in response to rapid changes in CO_2 levels. The levels of soluble foliar

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[2] Present address: Friedrich Miescher Institut, P.O. Box 2543, CH-4002, Basel, Switzerland.

* Corresponding author; e-mail coleman@botany.utoronto.ca; fax 1–416–978–5878.

Abbreviations: CA, carbonic anhydrase; *ca*, gene encoding carbonic anhydrase; C_i, inorganic carbon; *rbcS*, gene encoding the small subunit of Rubisco; RuBP, ribulose-1,5-bisphosphate.

carbohydrates were also determined in an effort to correlate changes in gene expression with leaf carbohydrate abundance.

MATERIALS AND METHODS

Seeds of pea (*Pisum sativum* cv Little Marvel) were grown in growth chambers at the appropriate CO_2 concentration for 3 weeks in complete soil, with a daylength of 16 h, PPFD of 400 μmol m^{-2} s^{-1}, and a day/night temperature regime of 20/16°C. Plants were watered daily and fertilized weekly with a commercial preparation (PlantProd 20–20–20 with micronutrients, Plant Products, Brampton, Ontairo, Canada). Pots were 20 cm in diameter with no more than three plants per pot. When required, plants were transferred to different CO_2 concentrations following 2.5 weeks of growth at high (1000 μL/L), ambient (350 μL/L), or low-CO_2 (160 μL/L) concentrations. Analyses were performed using the first set of fully expanded (mature) leaves or the set of developing (immature) leaves proximal to the apex.

Enzyme Activities

Leaves were excised from fully illuminated plants, their areas and fresh weights quickly determined, and then they were frozen in liquid nitrogen. When required, these frozen samples were ground with a mortar and pestle in extraction buffer (100 mM Bicine, pH 8.2, 20 mM $MgCl_2$, 5 mM DTT, 1 mM EDTA), and the samples were clarified by centrifugation for 10 min at 10,000*g* at 4°C. Total CA activity was determined electrometrically (Wilbur and Anderson, 1948). The rate of RuBP-dependent $^{14}CO_2$ incorporation at 25°C following full CO_2/Mg^{2+} activation of the enzyme was used to determine Rubisco activity (Hudson et al., 1992). Enzymes were assayed in triplicate and expressed on the basis of leaf area. Supernatant fractions were assayed for soluble protein (Bradford, 1976), and chlorophyll concentrations were determined (Porra et al., 1989).

Foliar Soluble Sugar Assays

Leaves from illuminated plants growing at the appropriate CO_2 concentrations were excised, their fresh weights and areas quickly determined, and then they were frozen in liquid nitrogen and ground in a mortar and pestle prior to perchloric acid extraction as described by Stitt et al. (1989). Foliar levels of Glc, Fru, and Suc were assayed using a coupled-enzyme system described previously (Stitt et al., 1989).

RNA Isolation and Northern Blot Hybridization

Isolation of total RNA from tissue harvested at the appropriate time was achieved using a previously described protocol (Majeau and Coleman, 1994). Equal aliquots of RNA (5 μg in each sample as determined spectrophotometrically) were denatured with formaldehyde:formamide (6.5:50%, v/v) and then immobilized on nitrocellulose using a slot-blot apparatus (Minifold II Slot-Blotter, Schleicher & Schuell) (Fourney et al., 1988). Prehybridization, hybridization, and probe-labeling protocols were all as described previously (Majeau and Coleman, 1994). Gene probes used were a 0.95-kb pea chloroplast *ca* cDNA (Majeau and Coleman, 1991), a 0.68-kb pea *rbcS* cDNA (Coruzzi et al., 1983), and an 18S soybean rDNA sequence used for normalization of RNA loads. Quantification of hybridization to pea RNA by all probes was achieved by phosphorimaging of the blots (model 400S, Molecular Dynamics, Sunnyvale, CA).

RESULTS

Growth of pea plants at different CO_2 concentrations resulted in major changes in leaf area and fresh weight, Rubisco and CA activity, and soluble protein content (Table I). High CO_2 levels (1000 μL/L) increased individual leaf area, as well as leaf fresh weight. In contrast, plants grown under low-CO_2 conditions (160 μL/L) had smaller leaves (both area and fresh weight) than control plants. In vitro Rubisco activity in the youngest, fully expanded leaves was measured following full activation of the enzyme. A greater than 35% reduction in activity (expressed on a leaf area basis) was found in leaves grown at high levels of CO_2 compared with air-grown plants. Growth of plants at low-CO_2 concentrations resulted in no significant decline in total activity. CA activity in high-CO_2-grown plants was reduced by 30%, whereas low-CO_2-grown plants exhibited an increase in CA activity of approximately 50% relative to air-grown plants.

To investigate the effects of different CO_2 growth conditions on *ca* and *rbcS* transcript abundance, total RNA was extracted from mature leaves (pairs of youngest, fully expanded leaves, as described in "Materials and Methods") of plants germinated and grown for 3 weeks at 160, 350, and 1000 μL/L CO_2. The results of the slot-blot analysis are shown in Figure 1. *ca* transcript levels for plants grown at high-CO_2 were reduced to less than 30% of that observed in plants grown in air; *rbcS* transcript levels were reduced in a similar fashion. Growth at 160 μL/L CO_2 also resulted in a decline in *ca* and *rbcS* transcript abundance below that

Table I. *Characteristics of the youngest, fully expanded leaves from pea plants grown at different CO_2 concentrations*

Values represent means ± SE of a minimum of six plant replicates from two separate experiments.

Characteristic	CO_2 Concentration		
	High (1000 μL/L)	Ambient (350 μL/L)	Low (160 μL/L)
Leaf fresh weight (mg)	98 ± 11	55 ± 14	20 ± 7
Leaf area (cm^2)	5.8 ± 0.8	4.3 ± 1.2	1.2 ± 0.2
Chlorophyll (mg m^{-2})	267 ± 23	251 ± 27	291 ± 30
Soluble protein (mg m^{-2})	2.05 ± 0.13	2.47 ± 0.44	3.09 ± 0.37
CA activity (units m^{-2} 10^{-5})	7.87 ± 1.18	11.39 ± 0.97	17.43 ± 1.22
Rubisco activity (μmol s^{-1} m^{-2})	33.2 ± 5.9	51.2 ± 5.5	49.1 ± 6.8

exhibited by the air-grown control plants, although the reduction in *ca* levels was less pronounced than that observed under high-CO_2 conditions (Fig. 1).

The effect on CA and Rubisco expression following transfer of pea plants from one CO_2 concentration to another was also examined. Plants grown for 2.5 weeks at high levels of CO_2 were transferred to ambient conditions, and their *ca* and *rbcS* transcript abundance in mature leaves determined (Fig. 2a). Both *ca* and *rbcS* transcript abundance exhibited a rapid increase following exposure to air, reaching maximum levels after 3 h. This was followed by a gradual decline, during which mRNA levels returned to the levels found in air-grown plants. Northern blot analysis of RNA isolated from immature leaves showed no changes in transcript abundance following transfer from high to air levels of CO_2 (data not shown); the effect on transcript abundance following transfer from air to high levels of CO_2 is shown in Figure 2b. Although the changes are less pronounced there appeared to be a decline in *ca* transcript abundance, with the lowest levels achieved at the 3-h time point, followed by a gradual increase to levels somewhat higher than those seen in high-CO_2-grown plants. Changes in *rbcS* transcript levels exhibited no discernible pattern, although the lowest value was obtained at the 3-h time point. Even after 12 h of exposure to 1000 μL/L CO_2, *ca* and *rbcS* transcript levels were still somewhat higher than those observed in high-CO_2-grown plants. RNA blot analysis of immature leaves did not reveal any significant changes in *rbcS* or *ca* transcript abundance during this time period (data not shown).

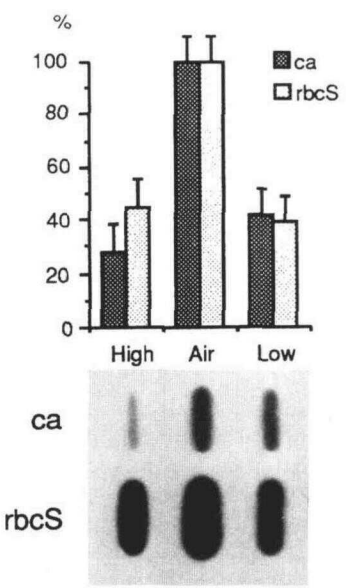

Figure 1. *ca* and *rbcS* transcript abundance of mature leaves grown at high (1000 μL/L), ambient (350 μL/L), and low (160 μL/L) concentrations of CO_2. Slot blots (5 μg of total RNA per slot) were probed with [^{32}P]dCTP-labeled cDNA encoding pea *ca* and *rbcS*. A radiolabeled soybean rDNA probe was used to ensure that each slot contained equal aliquots of pea RNA. The extent of probe hybridization was determined using phosphorimaging. Data are expressed as a percentage of the transcript level obtained for each gene from plants grown at air levels of CO_2 and represent the means ± SE of six sample replicates from two individual experiments. Leaf samples for RNA analysis were taken at the midpoint of the illumination period.

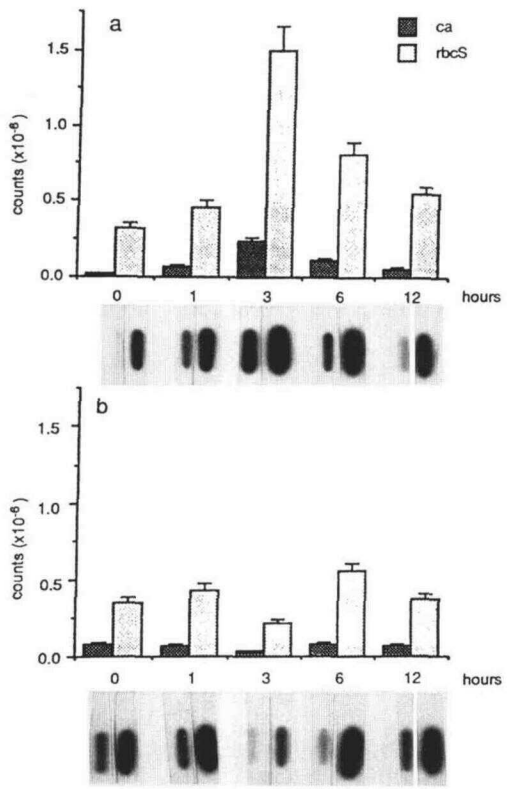

Figure 2. Time course of change in *ca* and *rbcS* transcript abundance following transfer of plants from 1000 to 350 μL/L CO_2 (a), and from 350 to 1000 μL/L CO_2 (b). Each slot contains 5 μg of total RNA and was probed with labeled cDNA encoding pea *ca* and *rbcS*, as well as with a soybean rDNA sequence for normalization of RNA loading. Data show the extent of probe hybridization (expressed as counts detected by phosphorimaging) to RNA in pooled extracts obtained from 15 individual plants and represent the means ± SE of three sample replicates.

CA and Rubisco activities were also examined in mature leaves acclimated for 24 h following transfer to a different CO_2 concentration (Table II). Exposure of high-CO_2-grown plants to a lower CO_2 concentration resulted in an increase in the activity of both enzymes, with levels approaching those observed in air-grown plants. Transfer of air-grown

Table II. *CA and Rubisco activities after transfer from high to ambient or low-CO_2 concentrations*

The youngest, fully expanded leaves were analyzed after 24 h of exposure to a lower CO_2 concentration. Values represent means ± SE of a minimum of four plant replicates from two experiments.

Characteristics	CO_2 Concentration		
	High (1000 μL/L)	Ambient (350 μL/L)	Low (160 μL/L)
CA activity (units m^{-2} 10^{-5})	7.17 ± 0.45	9.49 ± 1.79	10.65 ± 0.74
Rubisco activity (μmol s^{-1} m^{-2})	34.9 ± 4.6	42.6 ± 5.7	41.3 ± 6.4

plants to high-CO_2 concentrations for 24 h did not change CA or Rubisco activities in mature leaves (data not shown).

Levels of Fru/Glc and Suc were determined in the youngest, fully expanded leaf pairs obtained from plants grown at or transferred to various CO_2 concentrations (Table III). As expected, levels of Fru/Glc and Suc were highest in plants grown at 1000 μL/L CO_2. Transfer from high to air levels of CO_2 resulted in a significant decline in foliar Suc. Leaves from plants grown at air levels of CO_2 contained lower amounts of Fru/Glc and Suc. Suc levels increased rapidly following transfer from air to high levels of CO_2.

DISCUSSION

In this study we examined the effect of long- and short-term exposure to varying CO_2 concentrations on the expression of CA and Rubisco in pea leaves. Transcript abundance and enzymatic activity of both proteins decreased in mature leaves when plants were grown at a CO_2 concentration of 1000 μL/L. As recently reviewed by Bowes (1991) and Webber et al. (1994), there are many examples of exposure to high-CO_2 concentrations lowering both CA and Rubisco activities in C_3 plants. Lowering of Rubisco activity in response to elevated CO_2 has been ascribed to enzyme inactivation, inhibition of translation, and modulation of transcript abundance. In this study we show that down-regulation of both Rubisco and CA activity is accompanied by a reduction in *rbcS* and *ca* transcript levels. Recent studies have also shown that growth at high-CO_2 concentrations results in a decline in Rubisco activity (Xu et al., 1994) and transcript abundance (Riviere-Rolland et al., 1996) in pea. The latter study also indicated that high-CO_2 repression of Rubisco expression occurred only during growth at limiting N conditions. Although the N status of plants was not monitored during our study, the fertilization regime should have provided sufficient N, and no symptoms of N deficiency were observed. Cultivar variation in N utilization or allocation may account for the differences between the two studies.

In contrast to pea, in the only other published study (to our knowledge) on the CO_2 regulation of *ca* gene expression, increased *ca* transcript abundance was observed when *A. thaliana* plants were grown at 660 μL/L CO_2 (Raines et al., 1992). No measurements of activity or protein levels were reported, however, and it is not known if increased transcript levels resulted in changes in activity. The results from Arabidopsis may indicate that the control of CA expression by CO_2 concentration could vary between species, or that the lower CO_2 concentration used in the Arabidopsis study promotes CA expression. It has been reported that cotton grown at 660 μL/L CO_2 exhibits increased CA activity compared with air-grown plants, but growth at 1000 μL/L CO_2 represses CA activity (Chang, 1975). Plants grown at CO_2 concentrations below ambient also display a reduction in Rubisco activity, but have elevated levels of CA activity compared with air-grown plants. The decline in Rubisco activity, as well as modification of other leaf characteristics, are similar to those observed in the C_3 plant *Abutilon theophrasti* grown at 150 μL/L CO_2 (Tissue et al., 1994). Although CA levels were not measured in the *Abutilon* study, chlorophyll, Rubisco content and activity, and leaf mass were all reduced in plants grown at 150 μL/L compared with air-grown plants. In pea, *ca* transcript levels in mature leaves do not reflect enhanced enzyme activity, but are higher than those found in high-CO_2-grown plants. The difference between *ca* transcript abundance and activity may be a function of enhanced translational activity and/or a reduced rate of CA turnover under these growth conditions; these possibilities are under further study. Rubisco activity more closely parallels transcript abundance. Increased CA activity (which results in the CA/Rubisco ratio being enhanced) in plants grown at low-CO_2 levels may assist in the diffusion of C_i through the chloroplast and with HCO_3^- dehydration at the site of fixation.

Both *ca* and *rbcS* transcript levels of high-CO_2-grown plants increased significantly after a 3-h exposure to air levels of CO_2. There was also a significant increase in both CA and Rubisco activity 24 h after transfer from high-CO_2 to ambient or below-ambient levels of CO_2. It has recently been suggested that the modulation of nuclear-encoded photosynthetic gene expression by CO_2 is mediated by changes in the soluble carbohydrate concentration of the leaf (Stitt, 1991; van Oosten et al., 1994; van Oosten and Besford, 1995). High external CO_2 concentration results in the accumulation of elevated foliar levels of starch and soluble sugars when the rate of photosynthetic carbon fixation exceeds the translocation rate to sink tissues. Elevated levels of foliar carbohydrates are associated with reduced abundance of a number of transcripts encoding proteins required for carbon fixation (van Oosten et al., 1994). Similar results have been obtained by supplying

Table III. *Soluble carbohydrate content in leaves exposed to high or ambient levels of CO_2*

The youngest, fully expanded leaves were analyzed. Values represent means ± SE of eight plant replicates from one experiment. Similar results were obtained in replicate experiments.

Carbohydrate Concentration (mmol m^2)	High[a] (1000 μL/L)	CO_2 Concentration				
		Ambient[a]	High to ambient[b]		Ambient to high[b]	
		(350μL/L)	(3 h)	(24 h)	(3 h)	(24 h)
Glc and Fru	10.2 ± 3.6	6.6 ± 1.8	5.6 ± 1.3	6.4 ± 0.9	10.7 ± 1.3	9.7 ± 3.1
Suc	7.0 ± 1.5	1.7 ± 0.3	4.1 ± 1.1	2.8 ± 0.4	3.0 ± 0.5	7.5 ± 1.0

[a] Grown continuously at the indicated CO_2 concentration. [b] Transferred from growth CO_2 concentration to indicated CO_2 concentration and carbohydrate levels assayed after 3 or 24 h.

detached leaves with external sources of hexoses (Krapp et al., 1991, 1993; van Oosten et al., 1994). Convincing evidence for specific and coordinated hexose-dependent repression of transcription was provided in studies using reporter sequences fused to promoter regions obtained from maize photosynthetic genes (Sheen, 1990, 1994). In our study, the transfer of plants from high to air levels of CO_2 rapidly reduced the foliar levels of carbohydrates by decreasing photosynthesis but permitting continued translocation. The rapid increase in *ca* and *rbcS* mRNA levels could be a response to the removal of carbohydrate repression of transcriptional activity. The changes in *ca* and *rbcS* transcript abundance result in increases in enzyme activity following a 24-h exposure to air levels of CO_2.

The transfer of plants from air to high levels of CO_2 did not result in definitive changes in transcript abundance within the 12-h time course of the experiments. In our studies, it was only after long-term exposure to high levels of CO_2 that decreased CA and Rubisco expression was observed.

Immature pea leaves failed to down-regulate *ca* and *rbcS* transcript levels in response to elevated CO_2 concentrations. This observation is in agreement with the carbohydrate model of transcriptional regulation, as these immature tissues are effective sinks for carbohydrate reserves. In addition, the developmental stage of the nascent leaf may preclude modulation of gene expression by carbohydrate levels. In a recent publication by Nie et al. (1995), high-CO_2 (550 μL/L) field-grown wheat exhibited a similar down-regulation of a number of nuclear-encoded photosynthetic genes, but CO_2 modulation of gene expression was highly dependent on the stage of leaf and crop development. These data show that a number of additional factors, both environmental and developmental, may override carbohydrate regulation of gene expression in the field.

In our studies with pea, if we assume that elevated levels of foliar carbohydrates were responsible for the down-regulation of CA and Rubisco expression in high-CO_2-grown plants, this suggests that the source-to-sink allocation of photosynthate was limiting during these experiments. Although we attempted to maximize growth space (few plants per pot) and maintained the plants in a well-watered and fertilized environment, it is still possible that resource allocation to sinks such as roots was restricted by the physical environment. Further studies will be required to determine if pea translocation capacity is limited by biotic or abiotic factors when plants are grown at high-CO_2 concentrations.

In earlier studies we suggested that the expression of Rubisco and CA was coordinated and presumably controlled by similar mechanisms (Majeau and Coleman, 1994). Although this may be true with respect to developmental timing of expression, light regulation, and tissue specificity, it is apparent that in at least low-CO_2-grown plants, this coordination of expression can be broken. Although the mechanisms are dissimilar, these data are in agreement with antisense studies in which reductions in CA were not accompanied by a decline in Rubisco (Majeau et al., 1994; Price et al., 1994).

In conclusion, the data presented in this paper are generally consistent with the proposed model for carbohydrate regulation of photosynthetic gene expression in higher plants. It is also interesting to note that CA expression responds to below-ambient levels of CO_2 in a manner somewhat analogous to that observed in eukaryotic algae. The ability of the plant to rapidly modulate *ca* and *rbcS* transcript abundance in response to external CO_2 concentrations suggests that regulation of gene expression may be an important general mechanism in the acclimation of photosynthesis to changing environmental parameters.

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LITERATURE CITED

Badger MR, Price GD (1992) The CO_2 concentrating mechanism in cyanobacteria and green algae. Physiol Plant **84:** 606–615

Badger MR, Price GD (1994) The role of carbonic anhydrase in photosynthesis. Annu Rev Plant Physiol Plant Mol Biol **45:** 369–392

Bailly J, Coleman JR (1988) Effect of CO_2 concentration on protein biosynthesis and carbonic anhydrase expression in *Chlamydomonas reinhardtii*. Plant Physiol **87:** 833–840

Besford RT, Ludwig LJ, Withers AC (1990) The greenhouse effect: acclimation of tomato plants growing in high-CO_2, photosynthesis and ribulose-1,5-bisphosphate carboxylase protein. J Exp Bot **41:** 925–931

Bowes G (1991) Growth at elevated CO_2: photosynthetic responses mediated through Rubisco. Plant Cell Environ **14:** 795–806

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem **72:** 248–254

Cervigni T, Teofani F, Bassanelli C (1971) Effect of CO_2 on carbonic anhydrase in *Avena sativa* and *Zea mays*. Phytochemistry **10:** 2291–2294

Chang CW (1975) Carbon dioxide and senescence in cotton plants. Plant Physiol **55:** 515–519

Coleman JR (1991) The molecular and biochemical analyses of CO_2 concentrating mechanisms in cyanobacteria and green algae. Plant Cell Environ **14:** 861–867

Corruzi G, Broglie R, Cashmore A, Chua N-H (1983) Nucleotide sequences of two cDNA clones encoding the small subunit of ribulose-1,5-bisphosphate carboxylase and the major chlorophyll a/b binding thylakoid polypeptide. J Biol Chem **258:** 1399–1402

Fourney RM, Miyakoshi J, Day RS III, Patterson MC (1988) Northern blotting: efficient RNA staining and transfer. BRL Focus **10:** 5–7

Fukuzawa H, Fujiwara S, Yamamoto Y, Dionisio-Sese ML, Miyachi S (1990) cDNA cloning, sequence, and expression of carbonic anhydrase in *Chlamydomonas reinhardtii*: regulation by environmental CO_2 concentration. Proc Natl Acad Sci USA **87:** 4383–4387

Hudson GS, Evans JR, von Caemmerer S, Arvidson YBC, Andrews TJ (1992) Reduction of ribulose-1,5-bisphosphate carboxylase/oxygenase content by antisense RNA reduces photosynthesis in transgenic tobacco. Plant Physiol **98:** 294–302

Krapp A, Hofmann B, Schafer C, Stitt M (1993) Regulation of the expression of *rbcS* and other photosynthetic genes by carbohydrates: a mechanism for the "sink regulation" of photosynthesis? Plant J **3:** 817–828

Krapp A, Quick P, Stitt M (1991) Ribulose-1,5-bisphosphate carboxylase-oxygenase, other Calvin-cycle enzymes, and chlorophyll decrease when glucose is supplied to mature spinach leaves via the transpiration stream. Planta **186:** 58–69

Majeau N, Arnoldo MA, Coleman JR (1994) Modification of carbonic anhydrase activity by antisense and over-expression constructs in transgenic tobacco. Plant Mol Biol **25:** 377–385

Majeau N, Coleman JR (1991) Isolation and characterization of a cDNA coding for pea chloroplastic carbonic anhydrase. Plant Physiol **95:** 264–268

Majeau N, Coleman JR (1994) Correlation of carbonic anhydrase and ribulose-1,5-bisphosphate carboxylase/oxygenase expression in pea. Plant Physiol **104:** 1393–1399

Nie G, Hendrix DL, Webber AN, Kimball BA, Long SP (1995) Increased accumulation of carbohydrates and decreased photosynthetic gene transcript levels in wheat grown at an elevated CO_2 concentration in the field. Plant Physiol **108:** 975–983

Peet MM, Huber SC, Patterson DT (1986) Acclimation to high-CO_2 in moneoecious cucumbers. II. Carbon exchange rates, enzyme activities, and starch and nutrient concentrations. Plant Physiol **80:** 63–67

Porra RJ, Thompson WA, Kreidemann PE (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four solvents: verification of the concentration of chlorophyll standards by absorption spectroscopy. Biochim Biophys Acta **975:** 384–394

Porter MA, Grodzinski B (1984) Acclimation to CO_2 in bean. Carbonic anhydrase and ribulose bisphosphate carboxylase. Plant Physiol **74:** 413–416

Price GD, von Caemmerer S, Yu J-W, Lloyd J, Oja V, Kell P, Harrison K, Gallagher A, Badger MR (1994) Specific reduction of the chloroplast carbonic anhydrase activity by antisense RNA in transgenic tobacco has a minor effect on photosynthetic CO_2 assimilation. Planta **193:** 331–340

Raines CA, Horsnell PR, Holder C, Lloyd JC (1992) *Arabidopsis thaliana* carbonic anhydrase: cDNA sequence and effect of CO_2 on mRNA levels. Plant Mol Biol **20:** 1143–1148

Riviere-Rolland H, Contard P, Betsche T (1996) Adaptation of pea to elevated atmospheric CO_2: Rubisco, phosphoenolpyruvate carboxylase and chloroplast phosphate translocator at different levels of nitrogen and phosphorus nutrition. Plant Cell Environ **19:** 109–117

Rowland-Bamford AJ, Baker JT, Allen LH, Bowes G (1991) Acclimation of rice to changing atmospheric carbon dioxide concentration. Plant Cell Environ **14:** 577–583

Sage RF (1994) Acclimation of photosynthesis to increasing atmospheric CO_2: the gas exchange perspective. Photosynth Res **39:** 351–368

Sage RF, Sharkey TD, Seemann JR (1989) Acclimation of photosynthesis to elevated CO_2 in five C_3 species. Plant Physiol **89:** 590–596

Sheen J (1990) Metabolic repression of transcription in higher plants. Plant Cell **2:** 1027–1038

Sheen J (1994) Feedback control of gene expression. Photosynth Res **39:** 427–438

Stitt M (1991) Rising CO_2 levels and their potential significance for carbon flow in photosynthetic cells. Plant Cell Environ **14:** 741–762

Stitt M, Lilley R, Gerhardt R, Heldt HW (1989) Determination of metabolite levels in specific cells and subcellular compartments of plant leaves. Methods Enzymol **174:** 518–552

Sultemeyer D, Schmidt C, Fock HP (1993) Carbonic anhydrases in higher plants and aquatic microorganisms. Physiol Plant **88:** 179–190

Tissue DT, Griffin KL, Thomas RB, Strain BR (1994) Effects of low and elevated CO_2 on C_3 and C_4 annuals. II. Photosynthesis and biochemistry. Oecologia **101:** 21–28

van Oosten JJ, Besford RT (1995) Some relationships between the gas exchange, biochemistry and molecular biology of photosynthesis during leaf development of tomato plants after transfer to different carbon dioxide concentrations. Plant Cell Environ **18:** 1253–1266

van Oosten JJ, Wilkins D, Besford RT (1994) Regulation of the expression of photosynthetic nuclear genes by CO_2 is mimicked by regulation by carbohydrates: a mechanism for the acclimation of photosynthesis to high-CO_2? Plant Cell Environ **17:** 913–923

Webber AN, Nie GY, Long SP (1994) Acclimation of photosynthetic proteins to rising atmospheric CO_2. Photosynth Res **39:** 413–425

Wilbur KM, Anderson NG (1948) Electrometric and colorimetric determination of carbonic anhydrase. J Biol Chem **176:** 147–154

Xu D-Q, Gifford RM, Chow WS (1994) Photosynthetic acclimation in pea and soybean to high atmospheric CO_2 partial pressure. Plant Physiol **106:** 661–671