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Acclimation to High CO_2 in Bean[1]

CARBONIC ANHYDRASE AND RIBULOSE BISPHOSPHATE CARBOXYLASE

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ABSTRACT

Young bean plants (*Phaseolus vulgaris* L. cv Seafarer) grew faster in air enriched with CO_2 (1200 microliters per liter) than in ambient CO_2 (330 microliters per liter). However, by 7 days when increases in overall growth (dry weight, leaf area) were visible, there was a significant decline (about 25%) in the leaf mineral content (N, P, K, Ca, Mg) and a drop in the activity of two enzymes of carbon fixation, carbonic anhydrase and ribulose 1,5-bisphosphate (RuBP) carboxylase under high CO_2. Although the activity of neither enzyme was altered in young, expanding leaves during the acclimation period, in mature leaves the activity of carbonic anhydrase was reduced 95% compared with a decline of 50% in ambient CO_2. The drop in RuBP carboxylase was less extreme with 40% of the initial activity retained in the high CO_2 compared with 50% in the ambient atmosphere. While CO_2 enrichment might alter the flow of carbon into the glycolate pathway by modifying the activities of carbonic anhydrase or RuBP carboxylase, there is no early change in the ability of photosynthetic tissue to oxidize glycolate to CO_2.

Atmospheric CO_2 enrichment has been used to increase the productivity and yield of many crops (1, 10, 11, 17). Generally, CO_2 enrichment increases total dry weight and yield when light, temperature, and water are not limiting growth. Elevated CO_2 has the greatest impact on the plant organs developing during the treatment period (1, 10, 12). Experiments with soybeans (10) show that CO_2 enrichment only increases yield when applied during reproductive growth and not when applied during vegetative growth. Older leaves seem less responsive to high CO_2 than young leaves (1, 12), and differences between vegetatively growing plants in high and ambient CO_2 diminish with prolonged treatment (1). Leaf thickness increases with high CO_2 treatment (17) which may bias area-based photosynthetic measurements. In addition, the effect of atmospheric CO_2 on stomatal resistance varies by species (7, 11).

Several explanations of these phenomena have been proposed. The simplest is that the enhanced CO_2 availability increases the flow of carbon through the Calvin cycle and subsequently carbohydrate transport to growing tissue (13). During reproductive growth, the elevated carbohydrate supply may allow greater floral initiation and/or seed set (13). A second explanation suggests that high CO_2 suppresses photorespiratory carbon loss. Although the competitive effects of CO_2 and O_2 are well demonstrated at the enzymic level (18), competition effects at the leaf or plant level are more complicated (4, 20). Bravdo and Canvin (4) showed that photorespiratory rates were unaffected over a range of low to normal CO_2 concentrations. At CO_2 levels sufficient to saturate photosynthesis, a drop in O_2 concentration from 21% to less than 3% reduced net carbon fixation while at ambient CO_2 levels, the low O_2 concentration increased carbon fixation (20). Long term exposure to elevated CO_2 atmospheres has been shown to alter enzyme complements. In tomato, high CO_2 increases RuBP[2] carboxylase and depresses glycolic acid oxidase activities (12) while in cotton, RuBP carboxylase activity is suppressed by high CO_2 treatment (27).

When plants are grown in high CO_2, but assayed for net carbon fixation at ambient CO_2 concentrations, their photosynthetic rate is lower than that of plants grown in air (1, 7, 13, 17). When assayed in their growth environment (*i.e.* high CO_2), this effect is not observed. A similar result is commonly observed in algae grown in high CO_2 (15) and is attributed to the suppression of carbonic anhydrase in cells under high CO_2 (15, 24). One conclusion from the algal work is that carbonic anhydrase contributes to an increase in the cellular CO_2 in limited CO_2 environments (15, 23). Although the photosynthetic rate correlates well with changes in carbonic anhydrase during the acclimation of algal cell to ambient CO_2 (15), the relationship between carbonic anhydrase and photosynthesis has not been observed in higher plants (24). A reduced carbonic anhydrase level has been reported in cotton grown in high CO_2 (5). The experiments discussed below examine the effects of high and ambient CO_2 atmospheres on growth and composition of bean plants with particular reference to the activity of carbonic anhydrase, RuBP carboxylase, and glycolate oxidase during acclimation to high CO_2 growth conditions.

MATERIALS AND METHODS

Growing Conditions. Bean plants (*Phaseolus vulgaris* L. cv Seafarer) were grown in CO_2-enriched and ambient CO_2 atmospheres in individually controlled Plexiglas and polyethylene chambers placed in a glasshouse (21). Natural lighting was supplemented with high pressure sodium lamps providing a minimum light intensity of 400 $\mu E\ m^{-2}\ s^{-1}$ at leaf level as measured by a Lambda sensor. Day/night temperatures were set at 27/15°C. Air was circulated with air conditioners with approximately five complete air changes per hour in an open system. CO_2 was added to the airstream through a Matheson 8420 mass flow meter.

Bean seeds were germinated in vermiculite and transplanted 7 d after emergence into a 1:1:1 mixture of sand:vermiculite:peat and placed in the ambient atmosphere chamber. After a further 7 d, some of the plants were placed in the high CO_2 chamber. Growth continued for 7 or 14 d in either high (1200 $\mu l\ l^{-1}$) or ambient (330 $\mu l\ l^{-1}$) CO_2. Over the growing period, plants received half-strength Hoagland solution (14) three times weekly

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[2] Abbreviation: RuBP, ribulose 1,5-bisphosphate.

in addition to regular watering.

Enzyme Assays. Entire first true or trifoliate leaves for RuBP carboxylase and carbonic anhydrase assays were ground in 5 ml g^{-1} of 100 mM Tris-SO_4 (pH 8.2), 20 mM $MgSO_4$, 5 mM dithioerythritol with 0.5 g solid PVP using a chilled mortar and pestle. The homogenate was filtered through Miracloth and centrifuged 20 min at 20,000*g*. The pellet was discarded. Samples for glycolic acid oxidase assays were prepared similarly except that the extraction buffer was 100 mM K-phosphate (pH 7.5) with 0.5 g PVP.

Carbonic anhydrase (EC 4.2.1.1) was assayed by an electrometric method previously reported (23, 26). However, since the bean enzyme was found to require a thiol reagent, the assay buffer routinely contained 1 mM dithioerythritol.

Samples for assay of RuBP carboxylase (EC 4.1.1.39) were activated by addition of equal volumes of 100 mM Tris-SO_4 (pH 8.2), 20 mM $MgSO_4$, 10 mM dithioerythritol, and 20 mM $NaHCO_3$, and heating for 10 min at 50°C before assay (19).

Glycolic acid oxidase (EC 1.1.3.1) was assayed polarigraphically in a Hansatech O_2 electrode (9). The assay mixture contained 80 μmol sodium pyrophosphate adjusted to pH 8.5, 0.1 μmol flavin mononucleotide, 1 μmol NaN_3, 5 μmol K-phosphate, 0.2 mg protein sample, and 5 μmol glycolic acid in a total volume of 1 ml. As a further indication of glycolate oxidation activity in leaves acclimated to high CO_2, the rate of release of $^{14}CO_2$ from leaf discs incubated with [1-^{14}C]glycolate was estimated (9).

Leaf areas were determined with a Licor LI-3000 leaf area meter. Soluble protein was determined by a dye-binding method (3).

Dry weights were determined after 3-d drying at 75°C. Leaf mineral analyses were performed by the Soil and Plant Analysis Laboratory in the Land Resource Science Department at the University of Guelph using automated procedures for N, P, and K (25) and atomic absorption for Ca and Mg.

RESULTS

Growth and Composition Changes during CO_2 Enrichment. After 7 d, there was no visible effect of CO_2 enrichment on individual leaves but after 14 d, leaf area and dry weight had increased in the high CO_2 atmosphere (Table I). Not only did the high CO_2 treatment increase weight gain over the 14-d period, the net assimilation rate increased with prolonged treatment. Leaf-specific weight, soluble protein and Chl concentrations were not altered by CO_2 treatment after 7 d, but specific leaf weight was increased by 14 d in high CO_2.

Although the plants showed no visible signs of mineral deficiency after 7 d, mineral composition of the leaves was altered by CO_2 treatment. Leaves from high CO_2-grown plants contained approximately 75 and 65% of the control levels of N, P, K, Ca, and Mg after 7 and 14 d of treatment, respectively (Table II).

Table I. *Growth Parameters of Beans in High and Ambient Atmospheric [CO_2]*

Parameter	[CO_2]	Days of Treatment		
		0	7	14
Leaf area	Ambient	24.3	72.0	144.3
(cm^2 $plant^{-1}$)	High		89.0*	183.6*
Dry weight	Ambient	0.113	0.231	0.498
(g $plant^{-1}$)	High		0.305**	0.853**
Net assimilation rate	Ambient		0.0025	0.0037
(g cm^{-2} $week^{-1}$)	High		0.0029	0.0058
Specific leaf weight	Ambient	4.6	3.2	3.5
(mg cm^{-2})	High		3.4	4.6

*, significant at the 0.05 level.
**, significant at the 0.01 level.

Effect of CO_2 Enrichment on Carbonic Anhydrase and RuBP Carboxylase. The data in Table III show that leaf age (relative position) has a significant effect on enzyme activity and must be considered when assessing treatment effects. In bean, the oldest leaf had the lowest soluble protein, Chl content, and total activities for all three enzymes. However, the highest specific RuBP carboxylase activity was observed in the older leaves. A leaf of intermediate age (position 2) had the highest carbonic anhydrase specific activity.

After 7 d of CO_2 treatment, it is difficult to distinguish plants on the basis of size. At this time, there was no indication in young leaves of any difference in carbonic anhydrase or RuBP carboxylase activity (Fig. 1, A and B). The amount of soluble protein was also not affected by CO_2 treatment (Fig. 1C). However, in mature leaves, the levels of carbonic anhydrase (Fig. 1A) and RuBP carboxylase (Fig. 1B) declined markedly over the time course (*i.e.* 7 d) of the experiment regardless of the CO_2 treatment. The rate of loss of these enzymes was greater in the high CO_2-treated tissue. The total activity of both enzymes was unaffected by the CO_2 treatment in young leaves while in mature leaves, high CO_2 generally reduced enzyme activities but with greater variability within the treatments (data not shown).

After 4 d of treatment, 60% of the carbonic anhydrase was lost from high CO_2-grown leaves compared with 25% from air-grown leaves (Fig. 1A). By 7 d, 95% of the carbonic anhydrase activity was lost under high CO_2 while mature leaves in air retained 50% of the initial activity. In comparison, after 7 d of CO_2 enrichment, 40% of the initial RuBP carboxylase activity remained compared with 50% in air-grown tissue (Fig. 1B). Though the soluble protein content declined approximately 25% over the experimental period, atmospheric CO_2 treatment did

Table II. *Effect of High or Ambient CO_2 on Vegetative Mineral Composition*

Element	Treatment Concentrations			
	7		14	
	High	Ambient	High	Ambient
	%			
Nitrogen	2.96	3.98**	2.51	3.65**
Phosphorus	0.27	0.34**	0.23	0.35**
Potassium	2.31	2.85*	2.11	2.81*
Calcium	2.55	3.42*	2.26	3.62**
Magnesium	0.52	0.74*	0.53	0.88**

*, significant at the 0.05 level.
**, significant at the 0.01 level.

Table III. *The Effect of Leaf Position (Age) on Carbonic Anhydrase, Glycolic Acid Oxidase, and RuBP Carboxylase Activities*

Observations with the same letter (a or b) within a row cannot be distinguished with $\alpha = 0.05$.

Enzyme	Leaf Position (from Cotyledons)		
	1	2	3
Carbonic anhydrase			
Units mg^{-1} protein	113.9[a]	169.1[b]	134.6[a]
Units g^{-1} fresh wt	1573[a]	3774[b]	3563[b]
Glycolic acid oxidase			
μmol O_2 min^{-1} mg^{-1} protein	155.0[a]	150.8[a]	119.5[b]
μmol O_2 min^{-1} g^{-1} fresh wt	2461[a]	3676[b]	3109[b]
RuBP carboxylase			
nmol CO_2 min^{-1} mg^{-1} protein	26.0[a]	28.7[a]	20.6[b]
nmol CO_2 min^{-1} g^{-1} fresh wt	390[a]	595[b]	615[b]

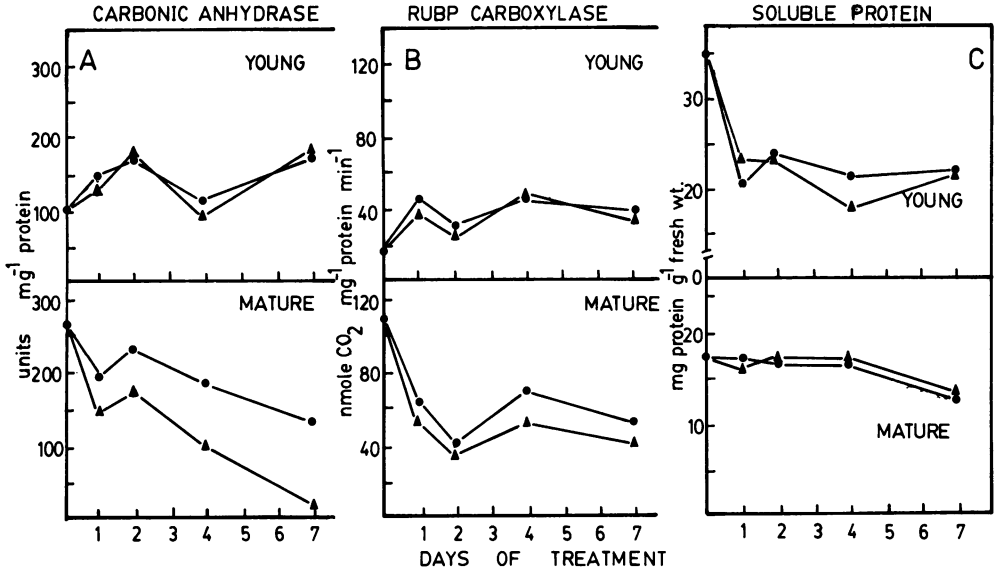

FIG. 1. The changes in carbonic anhydrase (A), RuBP carboxylase (B), and soluble protein (C) in young and mature leaves during acclimation to high CO_2. (● and ▲), the means of data (four replications) from ambient (●) and high (▲) CO_2-grown first trifoliate leaves, respectively.

Table IV. *Effect of CO_2 Enrichment on Glycolate Oxidation*

Parameter	[CO_2]	Oxidation
Glycolate oxidase		
μmol O_2 min^{-1} mg^{-1} protein	High	133
	Ambient	150
μmol O_2 min^{-1} g^{-1} fresh wt	High	3003
	Ambient	3161
CO_2 release from glycolate		
nmol h^{-1} 10 $discs^{-1}$	High	144
	Ambient	154

not affect the rate of decline.

CO_2 Enrichment and Glycolate Metabolism. Because CO_2 enrichment alters carbonic anhydrase and RuBP carboxylase activities which might affect the carboxylase/oxygenase activity ratio (2, 22) and thus glycolate synthesis (22) and/or glycolate oxidation (12), the effect of high CO_2 on glycolate oxidation was examined. Seven d of high CO_2 treatment did not change either the extractable glycolate oxidase activity or the ability of leaf discs to evolve CO_2 from exogenously supplied glycolate (Table IV) compared with air-grown tissue.

DISCUSSION

The study of young bean plants shows that CO_2 enrichment enhances growth rate (Table I) despite a reduced mineral content (Table II) and declining activities of carbon fixation enzymes in mature leaves (Fig. 1). On the basis of increased dry matter production and general size, the plants show no effects of CO_2 enrichment before about 1 week. Interestingly, before these effects are visible, there is a marked drop in the activities of carbonic anhydrase relative to RuBP carboxylase in mature leaves of plants grown in high CO_2. In C_3 plants, carbonic anhydrase is a chloroplast enzyme which catalyzes the interconversion of CO_2 and HCO_3^- (24). The significance of a reduced carbonic anhydrase activity during CO_2 enrichment is unclear, because the precise role of carbonic anhydrase in the stroma has not been defined (24). Carbon fixation in algae grown in high CO_2 is depressed when assayed in ambient CO_2, an effect attributed to suppression of carbonic anhydrase activity (15, 24). Perhaps in higher plants, less carbonic anhydrase is required because the CO_2 gradient between the atmosphere and the stroma is large enough to ensure high rates of photosynthesis. In this regard, it is the rate of loss of carbonic anhydrase which is markedly increased during the acclimation period to high CO_2 (Fig. 1A).

This observation may explain some paradoxical results reported previously (7, 13, 17). Plants grown in CO_2-enriched atmospheres grow faster, but photosynthesis of leaf tissue from these plants is 20 to 30% lower when measured at ambient CO_2 (~300 μl L^{-1}) than leaves grown in air. However, when measured at high CO_2 (1200 to 1500 μl L^{-1}), no difference in net photosynthesis is observed between leaves grown in air or CO_2-enriched atmospheres (13, 17). The greater loss of carbonic anhydrase activity (Fig. 1A) may explain the 'poorer' ability of high CO_2-acclimated plants to fix CO_2 efficiently at lower (ambient) CO_2 levels.

In vitro studies with partially purified RuBP carboxylase from wheat have demonstrated that the presence of carbonic anhydrase can lower the apparent K_m of the carboxylase for CO_2 (2). Similar studies with the spinach enzyme show that inhibition of added carbonic anhydrase reduces carboxylation and increases oxygenation of RuBP *in vitro* (22). Thus, suppression of carbonic anhydrase might allow higher oxygenation rates in high CO_2-acclimated plants by limiting the production of CO_2 from HCO_3^-. Alternatively, the role of carbonic anhydrase may lie in rapidly producing or consuming protons in the stroma-maintaining charge and/or ionic balance in the illuminated stroma (24). Loss of this rapid buffering capability might reduce carbon fixation in low (ambient) CO_2 in some yet unknown way.

The carboxylase is the predominant soluble leaf protein and the loss of its activity is not reflected in the loss of soluble protein (Fig. 1, B and C) as is often observed in senescing tissue (16). The slightly faster loss of RuBP carboxylase activity in high CO_2-grown tissue, compared with air-grown tissue is difficult to explain, but may also account for the lower net photosynthetic rates observed in high CO_2-acclimated plants assayed at ambient CO_2 levels (27).

The loss of either carbonic anhydrase or RuBP carboxylase might explain the reduced net photosynthesis of high CO_2-acclimated plants assayed in air, but the greatest effect would be expected when both decline as described above.

The data in Table IV indicate that there was no rapid loss in glycolate metabolizing capability, somewhat in contrast to Hinklenton and Jolliffe (12) who showed decreased glycolate oxidase activity after prolonged exposure to high CO_2. The amount of extractable glycolate oxidase and the ability of the leaf tissue to take up and metabolize exogenous glycolate was unchanged by

CO_2 enrichment. Similarly, *Chlorella* grown under high CO_2 temporarily excretes glycolate when transferred to low CO_2 even though the cells actually can metabolize more glycolate than air-grown cells (6). Bravdo and Canvin (4) suggest that higher plant photorespiration occurs as rapidly at high CO_2 as at ambient CO_2. We suggest that if higher plants alter photorespiratory activity in high CO_2, the main effect is in the control of carbon flow into the glycolate pathway and not in the glycolate pathway itself. In this regard, changes in the carbonic anhydrase and RuBP carboxylase levels in mature photosynthetically active leaves may be significant.

CONCLUSION

Carbonic anhydrase and RuBP carboxylase activities in young bean leaves are not rapidly changed by acclimation to high CO_2. In mature leaves, activities of both enzymes fall during the treatment period with activities of both enzymes declining faster in the high CO_2 treatment. The differential loss of these two enzymes might explain the lower net photosynthetic rates commonly observed in leaves acclimated to high CO_2 but assayed in ambient CO_2.

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