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Acclimation of Two Tomato Species to High Atmospheric CO_2[1]

II. Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase and Phospho*enol*pyruvate Carboxylase

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ABSTRACT

***Lycopersicon esculentum* Mill. cv Vedettos and *Lycopersicon chmielewskii* Rick, LA 1028, were exposed to two CO_2 concentrations (330 or 900 microliters per liter) for 10 weeks. The elevated CO_2 concentrations increased the initial ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity of both species for the first 5 weeks of treatment but the difference did not persist during the last 5 weeks. The activity of Mg^{2+}-CO_2-activated Rubisco was higher in 900 microliters per liter for the first 2 weeks but declined sharply thereafter. After 10 weeks, leaves grown at 330 microliters per liter CO_2 had about twice the Rubisco activity compared with those grown at 900 microliters per liter CO_2. The two species showed the same trend to Rubisco declines under high CO_2 concentrations. The percent activation of Rubisco was always higher under high CO_2. The phospho*enol*pyruvate carboxylase (PEPCase) activity measured in tomato leaves averaged 7.9% of the total Rubisco. PEPCase showed a similar trend with time as the initial Rubisco but with no significant difference between nonenriched and CO_2-enriched plants. Long-term exposure of tomato plants to high CO_2 was previously shown to induce a decline of photosynthetic efficiency. Based on the current study and on previous results, we propose that the decline of activated Rubisco is the main cause of the acclimation of tomato plants to high CO_2 concentrations.**

CO_2 enrichment is widely used to increase the growth of many greenhouse species. Compared with 340 μL L^{-1} CO_2, a significant increase in photosynthesis can be expected at 1000 μL L^{-1} CO_2 (12). Many studies have reported that the most beneficial effects of CO_2 enrichment occurred during the early stages of growth. Thereafter, plants acclimate to high CO_2 concentrations and gradually lose photosynthetic efficiency.

In a recent paper (23), we reported that the initial beneficial effects of CO_2 enrichment on the relative growth and the photosynthetic rates of tomato were not maintained as the plants matured. Many studies have quantified this long-term decline (7, 11, 12), yet there is still no consensus on the causes of this phenomenon. Elsewhere (23), we concluded that the decrease of stomatal conductance could not totally account for the acclimation of tomato plants to high CO_2. We also showed that the accumulation of starch and sugars and the modification of chloroplast ultrastructure were not the primary causes of declining photosynthesis (24).

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco)[2] is the major enzyme regulating the photosynthetic carbon assimilation in plants (10, 13). Many studies have reported a correlation between the decline of photosynthesis and the decrease in Rubisco with extended use of CO_2 enrichment (12, 14, 18). However, since many other parameters were also altered, no studies have yet been able to single out the primary cause of acclimation to high CO_2.

Previously, we showed that *Lycopersicon esculentum* and *L. chmielewskii* acclimated similarly to high atmospheric CO_2 (23), even though they differ significantly in their sink metabolism, carbohydrate assimilation (22), and starch and sugar contents under high CO_2 (24). We concluded from the latest study that the decline in photosynthesis of *L. esculentum* was not caused by a buildup of starch. However, the results were not as evident for *L. chmielewskii*. The objective of the current study was to explain the acclimation of tomato plants to high atmospheric CO_2 in relation to the activities of Rubisco and PEPCase in both tomato species.

MATERIALS AND METHODS

Plant Material

Lycopersicon esculentum Mill. cv Vedettos, and *Lycopersicon chmielewskii* Rick, LA 1028, were seeded in rockwool blocks (Grodania, Denmark) on December 15, 1987 and transplanted on January 17, 1988. Treatments consisted of two CO_2 concentrations: 330 ± 50 (control) or 900 ± 50 μL L^{-1}. Each treatment was repeated twice. The plants were grown as described previously (24).

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[2] Abbreviations: Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; PEPCase, phospho*enol*pyruvate; RuBP, ribulose-1,5-bisphosphate.

Rubisco and PEPCase

Rubisco and PEPCase were measured weekly for 10 weeks on leaf 5 from four plants randomly sampled from each experimental unit. Samples were ground in liquid nitrogen with 1% PVP-40 (wt/fresh wt tissue) and then homogenized with the extraction solution (100 mM bicine, 5 mM sodium ascorbate, 5 mM DTT, and 1.0 mM NaEDTA, pH 8.1). One mL was removed for determination of Chl content while 6 mL were centrifuged at 15,000*g* for 12 min at 2°C. The activity of Mg^{2+}-CO_2-activated Rubisco was determined by combining 1 mL of the supernatant with 1 mL of activation solution (100 mM bicine, 20 mM $MgCl_2$, and 20 mM $NaHCO_3$, pH 8.1) and incubating at room temperature for 20 min. The assay solution consisted of 100 mM bicine, 5 mM DTT, 0.4 mM ribulose 1,5-biphosphate, 0.1 mM NaEDTA, 20 mM $MgCl_2$, and 10 mM $NaH^{14}CO_3$ (10 mmol/mCi; DuPont-New England Nuclear, Boston, MA), pH 8.1, at a final volume of 1.0 mL. The assay ran for 2 min at 25°C. It was started by injection of 0.1 mL of the activated supernatant and stopped by the addition of 65 μL N HCl. The initial Rubisco activity was measured by injecting 0.1 mL of supernatant into the assay solution as described above. The vials were then dried and counted with a liquid scintillation counter. Each sample was assayed in duplicate.

The PEPCase assay used a procedure similar to that used to measure the initial Rubisco, but the reaction solution was different. The reaction solution for the PEPCase assay consisted of 100 mM bicine, 10 mM $MgCl_2$, 5 mM DTT, 0.1 mM NaEDTA, 5 mM sodium glutamine, 5 mM phospho*enol*pyruvate, and 10 mM $NaH^{14}CO_3$ (10 mmol/mCi, DuPont-New England Nuclear, Boston, MA) at a pH of 8.1. The PEPCase assay employed the same leaf extracts used for the Rubisco assays.

RESULTS AND DISCUSSION

The initial Rubisco activity of the leaf 5 of *L. esculentum* and *L. chmielewskii* was higher for 900 μL L^{-1} CO_2-grown plants for the first 5 weeks of treatment but the difference did not persist during the last 5 weeks (Fig. 1). At week 1, *L. esculentum* plants grown under CO_2-enriched conditions had rates of initial Rubisco activity that were 28.7% and 62.7% higher at 0800 and 1600 h, respectively. The corresponding values for *L. chmielewskii* were 41.9% and 79.5%. The larger differences observed in initial Rubisco rates of *L. chmielewskii* were related to the photosynthetic rates previously reported (23, 24). Such a result was to be expected since the initial activity of Rubisco is directly related to the leaf photosynthetic rate (17). Accordingly, the faster rate of decline of initial activity of high CO_2-grown plants compared with the control plants was correlated to the decline of photosynthesis previously reported for these plants (23, 24). The differences between treatments in initial Rubisco activity during the first 5 weeks were more pronounced at the afternoon than at the morning sampling for both species. In a previous paper (24), we reported that starch had built up during the day for both CO_2 concentrations, but the accumulation was more pronounced at 900 than 330 μL L^{-1} CO_2. These results suggest that the greater buildup of starch throughout the day in the

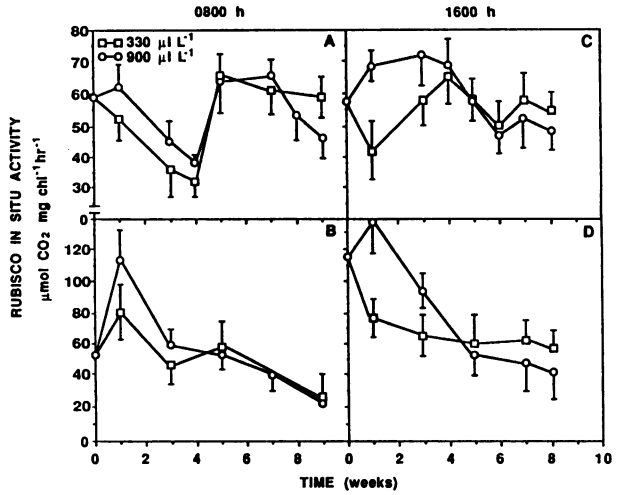

Figure 1. Initial Rubisco activity for the fifth (top to bottom) leaf of tomato plants grown at 330 and 900 μl·L^{-1} CO_2 for a 10 week period. A and C, *L. esculentum* at 0800 h and 1600 h; B and D, *L. chmielewskii* at 0800 h and 1600 h. Each point represents the mean of four values ± SE.

900 μL L^{-1} CO_2-grown plants did not cause feedback inhibition of initial Rubisco activity since the difference in initial Rubisco activity between the two CO_2 treatments was greater at the afternoon than at the morning sampling.

The absolute values of initial Rubisco sampled at 0800 h were significantly higher for *L. chmielewskii* than for *L. esculentum* at week 1. For the afternoon samplings, initial activity was higher in *L. chmielewskii* than in *L. esculentum* for the first 3 weeks. These results suggest that *L. chmielewskii* has a higher photosynthetic capacity than *L. esculentum* during the early stages of growth. This wild species may thus offer some interesting photosynthetic characteristics for further study.

The Mg^{2+}-CO_2-activated Rubisco activity of the fifth leaf of *L. chmielewskii* was higher in 900 μL L^{-1} CO_2-grown plants for the first 2 weeks but declined sharply thereafter (Fig. 2). *L. esculentum* showed a similar Mg^{2+}-CO_2-activated Rubisco activity at both CO_2 concentrations for the first 2 weeks, but declined at a rate similar to *L. chmielewskii* thereafter. After 10 weeks, leaves grown at 330 μL L^{-1} CO_2 had about twice the Rubisco activity of those grown at 900 μL L^{-1} CO_2. Peet *et al.* (12), Porter and Grodzinski (14), and Spencer and Bowes (18) also found a significant decline of activated Rubisco with CO_2 enrichment. For the first 3 weeks of treatment, the absolute values of Mg^{2+}-CO_2-activated Rubisco sampled at 1600 h were higher for *L. chmielewskii* than for *L. esculentum*. These results provide additional support to the idea that *L. chmielewskii* has a higher photosynthetic capacity than *L. esculentum* during the early stages of development.

Activated Rubisco activities were the same at 0800 h and 1600 h for *L. esculentum*. For *L. chmielewskii*, there was a further activation of the enzyme from 0800 h to 1600 h (Fig. 2 B, D). Besford (2) showed that the Mg^{2+}-CO_2-activated form of tomato Rubisco was present in smaller amounts in the morning even when the enzyme was activated and assayed at saturating levels of Mg^{2+}-CO_2. He showed that Rubisco re-

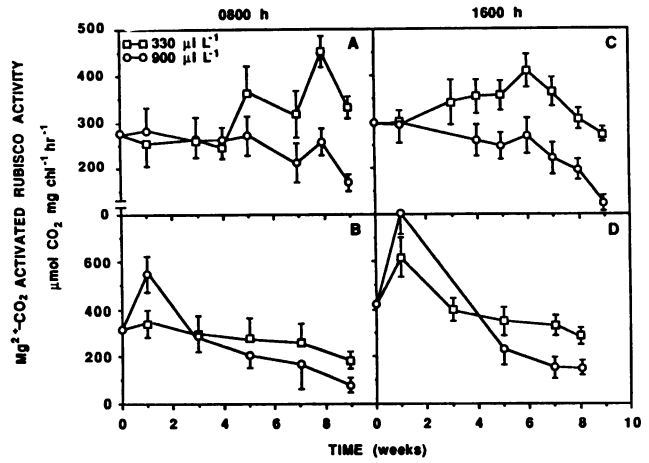

Figure 2. Mg^{2+} CO_2 activated Rubisco activity for the fifth (top to bottom) leaf of tomato plants grown at 330 and 900 $\mu l \cdot L^{-1}$ CO_2 for a 10 week period. A and C, *L. esculentum* at 0800 h and 1600 h; B and D, *L. chmielewskii* at 0800 h and 1600 h. Each point represents the mean of four values ± SE.

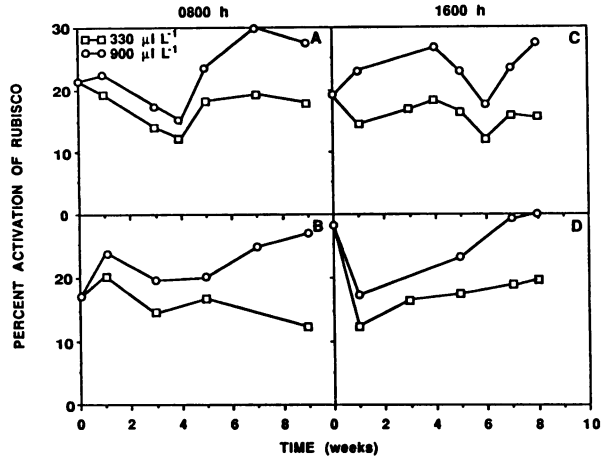

Figure 3. Percent activation of Rubisco for the fifth (top to bottom) leaf of tomato plants grown at 330 and 900 $\mu l \cdot L^{-1}$ CO_2 for a 10 week period. A and C, *L. esculentum* at 0800 h and 1600 h; B and D, *L. chmielewskii* at 0800 h and 1600 h. Each point represents the mean of four values ± SE.

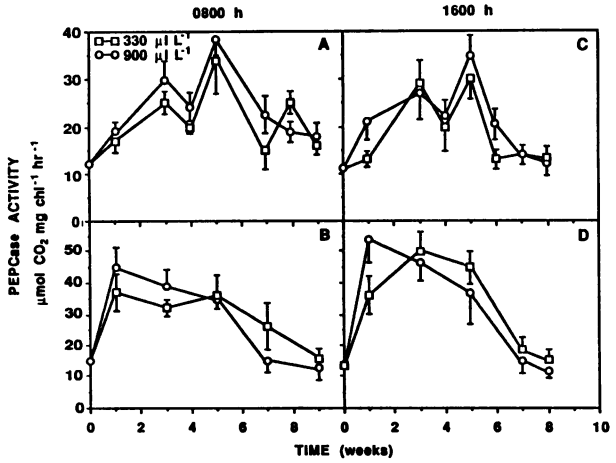

Figure 4. PEPCase activity for the fifth (top to bottom) leaf of tomato plants grown at 330 and 900 $\mu l \cdot L^{-1}$ CO_2 for a 10 week period. A and C, *L. esculentum* at 0800 h and 1600 h; B and D, *L. chmielewskii* at 0800 h and 1600 h. Each point represents the mean of four values ± SE.

quired 2.5 h of light before reaching its maximum activity, after which the activated form was stable throughout the day. Vu *et al.* (20) also reported that light affected the activated pool of Rubisco in soybean leaves. They hypothesized that, at night, a dark inhibitor bound and inactivated Rubisco, whereas, in presence of light, inhibition did not occur. Salvucci *et al.* (16) demonstrated that Rubisco activase was necessary to preactivate Rubisco. The preactivated form was then activated by CO_2 and Mg^{2+}. In our experiment, the low rates of initial Rubisco are due to the low light levels under which the plants were harvested. Even with the added supplemental lighting (150 μmol/m^2 s), we did not have more than 250 to 300 μmol/m^2 s on the leaves sampled at 0800 and 1600 h.

Throughout the experiment, the initial form of Rubisco was consistently lower than the activated form, indicating the potential for higher rates of photosynthesis in the leaves. These results suggest that increased light intensity could have increased net leaf photosynthesis. The percentage of activation of Rubisco (initial/Mg^{2+}-CO_2-activated × 100) was altered by CO_2 enrichment (Fig. 3). At 900 μL L^{-1} CO_2, the average percentages of activation were 22.4% and 23.2% for *L. esculentum* and *L. chmielewskii*, respectively. The corresponding values at 330 μL L^{-1} CO_2 were 17.1% and 17.2%. The percentage of functional enzyme was always higher (an average of 33% higher) under high CO_2 levels. The difference between the percentage of activation of CO_2-enriched and nonenriched plants increased gradually throughout plant growth for leaf 5 of both species sampled at 0800 h, but the difference was less pronounced for leaf 5 sampled at 1600 h. The percentage of activation of Rubisco for high CO_2-grown plants did not show any decline throughout the experiment. We postulate that the acclimation of tomato plants to high CO_2 cannot be attributed to a deficiency in the activation of Rubisco. Since the high CO_2 concentration did not affect the chlorophyll level of leaf 5 (data not shown), the decrease of activated Rubisco seems more likely to explain the long-term acclimation. Our data suggest that the beneficial effects of high CO_2 on the inhibition of photorespiration and activation of ribulose-1,5-bisphosphate carboxylase were more than offset by the decrease of activated Rubisco, thus resulting in the long-term decline of photosynthesis of high CO_2-grown plants.

The PEPCase activity measured in tomato leaves (Fig. 4) averaged 7.9% of the total Rubisco activity. Thus it can be concluded that Rubisco was the primary carboxylation enzyme for tomatoes. According to Zima and Sestak (25) and Raghavendra (15), PEPCase could account for a potential parallel CO_2 fixing pathway by young leaves of C_3 species. Besford *et al.* (3) reported that PEP/HCO_3-activated PEPCase might be responsible for some of the CO_2 assimilation of the youngest leaves of tomato. However, consideration of PEPCase in the calculation of CO_2 assimilation of mature tomato leaves led to an overestimation of photosynthetic rates (3). PEPCase showed a similar trend with time as initial Rubisco

but with no significant difference between nonenriched and enriched plants (Fig. 4). These results suggest that CO_2 affects the pool of Rubisco more than the pools of PEPCase. Acclimation appears to be a result of regulation at the genetic level for the Rubisco enzymes. Bailly and Coleman (1) reported that external CO_2 concentrations caused a change in carbonic anhydrase mRNA of green alga. The same mechanism may occur with Rubisco for tomato leaf exposed to high atmospheric CO_2.

CONCLUSIONS

Tomato plants grown under high atmospheric CO_2 concentrations had higher initial activities of Rubisco only in the early stages of plant development. The variations in relative growth rate (23) and carbon exchange rate (23, 24) followed the variations in initial Rubisco activity. However, even though initial Rubisco activities of enriched plants decreased below the controls, the photosynthetic rates of the enriched plants were never significantly lower than the controls (23, 24). These results suggest that the mechanism of increased photosynthetic rates of plants exposed to elevated atmospheric CO_2 resulted from increased RuBP carboxylation and decreased RuBP oxygenation.

The higher percent activation of Rubisco under high CO_2 level indicates that the long-term decline of photosynthesis throughout the experiment cannot be attributed to a deficiency in the activation of Rubisco. Our results suggest that it was not a modification in the concentration of Mg^{2+} or in pH that caused the acclimation. Von Caemmerer and Farquhar (19) calculated that high intercellular CO_2 levels could reduce RuBP regeneration, thereby limiting assimilation. The long-term decline of photosynthesis at high CO_2 level could result from a reduction of the electron chain transport capacity to supply ATP and NADPH to re-generate RuBP. However, Vu *et al.* (20) found that high atmospheric CO_2 increased RuBP levels but decreased Rubisco activity. Dietz and Heber (5) also suggested that RuBP was not a limiting factor under high CO_2 concentration. In a previous experiment (21), we showed that high photosynthetic photon flux density, which should increase the level of RuBP, did not attenuate the long-term decline of photosynthesis of CO_2-enriched plant. Thus a limitation in RuBP generation does not appear to be the limiting factor.

We propose that the decline of activated Rubisco is the main cause of the acclimation of tomato plants to high CO_2 concentration. We previously showed that stomatal conductance declined significantly under high atmospheric CO_2 (23). However, this decline could not by itself explain the reduced photosynthetic rate of these plants since internal CO_2 concentrations remained constant during plant growth. We also measured a significant accumulation of starch and sugars in leaf 9 grown under high atmospheric CO_2 concentrations. This buildup resulted in disruption of the thylakoids in some cases. However, we did not find any major accumulation of sugar or starch in leaf 5, which showed decline in photosynthesis equal to that of leaf 9. Therefore, starch accumulation appears to be a symptom, but not the primary cause, of the loss of photosynthetic efficiency at high CO_2 concentrations. Nevertheless, starch accumulation can cause feedback inhibition and then enhance the loss of efficiency of high CO_2-grown plants.

The effect of high CO_2 on the lowering of activated Rubisco can be at two levels: (a) the decrease of Rubisco protein or (b) the presence of a specific inhibitor that binds to the enzyme under high CO_2, and then causes an incomplete activation of the enzyme. We found a 50% reduction in the Mg^{2+}-CO_2-activated Rubisco rate after 10 weeks of enrichment. Since light levels were gradually increasing throughout the course of the experiment, we conclude that the diminished activity is directly related to the enzyme content. The effect of high CO_2 enrichment on protein content appears specific to Rubisco. PEPCase as well as nitrate reductase activates were not significantly affected by CO_2 enrichment. The modification of the content of the Rubisco protein could be attributed to a decrease of synthesis or an increase of degradation (protein breakdown) of the enzyme. We propose the decreased synthesis as a more probably mechanism. Plants grown at high CO_2 concentration accumulated high levels of carbohydrates in the leaves with time (24). This implied a high carbohydrate status for these plants. Under these conditions, maintenance of Rubisco enzyme synthesis at pre-enriched levels becomes energetically wasteful and may be limited by nitrogen availability. Thus, with time, the Rubisco contents declines until a new homeostatic equilibrium is established.

Vu *et al.* (20) stated that C_4 plants that possess PEPCase to concentrate internal CO_2 have naturally lower levels of Rubisco protein than do C_3 plants. These results imply that the effects of high CO_2 levels on the concentration of Rubisco protein of C_3 plants may be analogous in both C_3 and C_4 plants. Recent studies showed that protein synthesis was affected by high CO_2 level. Bailly and Coleman (1) reported that high CO_2 levels control gene expression of carbonic anhydrase in green alga. However, no studies have demonstrated high atmospheric CO_2 control of gene expression of Rubisco.

The second hypothesis to explain the low level of activated Rubisco in the presence of inhibitors that bind to the protein. Loomis (9) found that the activity of polyphenol oxidases caused the inactivation of many enzymes when phenols were present. The polyphenol oxidases were associated with the thylakoid membrane (4). Koivuniemi *et al.* (8) established a correlation between the decrease of Rubisco activity and the increase of polyphenol oxidase activity in tobacco leaf extracts. However, Downton *et al.* (6) showed that the reduction of Rubisco activity of *Nerium oleander* grown under high CO_2 levels was associated with a reduced amount of Rubisco protein. Thus further research is required to determine how and why Rubisco is affected by high atmospheric CO_2.

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