Plant Physiol. (1997) 115: 1569–1580

Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase Activase Deficiency Delays Senescence of Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase but Progressively Impairs Its Catalysis during Tobacco Leaf Development[1]

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Transgenic tobacco (*Nicotiana tabacum* L. cv W38) plants with an antisense gene directed against the mRNA of ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) activase grew more slowly than wild-type plants in a CO_2-enriched atmosphere, but eventually attained the same height and number of leaves. Compared with the wild type, the anti-activase plants had reduced CO_2 assimilation rates, normal contents of chlorophyll and soluble leaf protein, and much higher Rubisco contents, particularly in older leaves. Activase deficiency greatly delayed the usual developmental decline in Rubisco content seen in wild-type leaves. This effect was much less obvious in another transgenic tobacco with an antisense gene directed against chloroplast-located glyceraldehyde-3-phosphate dehydrogenase, which also had reduced photosynthetic rates and delayed development. Although Rubisco carbamylation was reduced in the anti-activase plants, the reduction was not sufficient to explain the reduced photosynthetic rate of older anti-activase leaves. Instead, up to a 10-fold reduction in the catalytic turnover rate of carbamylated Rubisco in vivo appeared to be the main cause. Slower catalytic turnover by carbamylated Rubisco was particularly obvious in high-CO_2-grown leaves but was also detectable in air-grown leaves. Rubisco activity measured immediately after rapid extraction of anti-activase leaves was not much less than that predicted from its degree of carbamylation, ruling out slow release of an inhibitor from carbamylated sites as a major cause of the phenomenon. Nor could substrate scarcity or product inhibition account for the impairment. We conclude that activase must have a role in vivo, direct or indirect, in promoting the activity of carbamylated Rubisco in addition to its role in promoting carbamylation.

Rubisco (EC 4.1.1.39) is a key regulatory enzyme of photosynthetic CO_2 assimilation. To become catalytically competent, Rubisco must be activated through carbamylation of an active-site Lys residue to allow binding of the catalytically essential divalent metal ion (for review, see Andrews and Lorimer, 1987; Hartman and Harpel, 1994). The carbamylation process can be modulated by a variety of regulators, such as RuBP (Jordan and Chollet, 1983), CA1P (Vu et al., 1984; Seemann et al., 1985; Servaites, 1990), and some by-products of the RuBP carboxylation reaction (Edmondson et al., 1990; Zhu and Jensen, 1991).

In chlorophytes another chloroplast protein, activase, mediates the activation of Rubisco. Activase maintains Rubisco activity by facilitating the release of inhibitors that impede carbamylation or catalysis (Portis, 1992). It was first identified by analysis of the *rca* mutant of *Arabidopsis thaliana*, which required CO_2 supplementation for growth (Somerville et al., 1982; Salvucci et al., 1985). We (Mate et al., 1993, 1996; Andrews et al., 1995) and Jiang et al. (1994) have used antisense-RNA technology to generate transgenic tobacco plants with reduced activase contents, and a similar strategy has also been applied to *A. thaliana* (Eckardt et al., 1997). Our plants have <1 to 20% of the activase content of wild-type plants. These plants have impaired photosynthetic capacity and some cannot grow without CO_2 supplementation. Even some of those that survive without CO_2 supplementation have reduced CO_2 assimilation rates, which correlate with reduced Rubisco carbamylation (Mate et al., 1996). CA1P release from Rubisco is retarded in these plants (Mate et al., 1993), supporting the idea that activase facilitates the dissociation of such ligands (Robinson and Portis, 1988). Tobacco plants with more than 20% of the wild-type activase content appear little different from the wild type (Jiang et al., 1994).

Photosynthetic capacity during leaf ontogeny in dicotyledons has usually been divided into three phases: an increasing period coincident with leaf expansion, a maximal phase, and a senescence period (Gepstein, 1988). A variety of factors are involved in controlling photosynthetic rate during leaf development. One of the obvious characteristics of late leaf development and of senescence is the loss of proteins; Rubisco, especially, accounts for a major portion of the loss in most C_3 species (Huffaker,

[1] This work was supported by an Australian National University Scholarship and the Cooperative Research Centre for Plant Science (Z.H.).

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Abbreviations: Activase, Rubisco activase; CA1P, 2′-carboxyarabinitol-1-phosphate; CPBP, unresolved isomeric mixture of 2′-carboxyarabinitol-1,5-bisphosphate and 2′-carboxyribitol-1,5-bisphosphate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PGA, 3-phospho-D-glycerate; RuBP, D-ribulose-1,5-bisphosphate.

1990). However, leaf-development programs were not seriously affected by reductions in Rubisco content in transgenic tobacco plants with an *rbc*S antisense gene (Jiang and Rodermel, 1995). The effect of activase deficiency on leaf developmental processes has not been studied.

Prompted by our earlier observations that the anti-activase plants sometimes contained considerably larger amounts of Rubisco than control plants (Mate et al., 1993; Andrews et al., 1995), we explored how decreases in the amount of activase influence contents of soluble protein and Rubisco, Rubisco carbamylation and activity, and photosynthetic capacity during leaf and plant development. We grew plants with <15% of the activase content of the wild type with CO_2 supplementation to allow them to complete a relatively normal life cycle. Another transgenic tobacco line with a similar reduction in photosynthetic capacity caused by reduced chloroplastic GAPDH activity (Price et al., 1995) was used for comparison. We discovered that activase deficiency postponed the decline in Rubisco content seen in wild-type leaves as they develop, and that the large amount of Rubisco remaining in older anti-activase leaves was inactivated to a much greater extent than its carbamylation status predicted. Although carbamylated but catalytically impaired Rubisco was most obvious in old leaves grown in high CO_2, it could also be detected in younger leaves and in leaves from plants grown without CO_2 supplementation. In another experiment, we compared the activity of Rubisco in vivo with that measured in vitro after rapid extraction of the leaves. Even with very rapid extraction directly into the assay medium, initial Rubisco activity was close to that predicted from the carbamylation status regardless of genotype, leaf age, or growth conditions.

MATERIALS AND METHODS

Plant Material and Growth

Plants used were from the R1 progeny of *Nicotiana tabacum* L. cv W38 transformed with either a T-DNA from pαTACT, containing an antisense gene directed against activase (Mate et al., 1993), or a T-DNA from pBIN-GAP, containing an antisense gene directed against GAPDH (Price et al., 1995). The anti-activase plants were the progeny of primary transformant A52, which had two T-DNA inserts (Mate et al., 1993); the anti-GAPDH plants were the progeny of GAP-R, also having two T-DNA inserts (Price et al., 1995). Wild-type cv W38 was used as the control. Plants were grown in sterilized garden soil in 5-L pots in growth cabinets in atmospheres containing 1500 or 350 to 400 μbar CO_2 at an irradiance of 450 to 550 μmol quanta m^{-2} s^{-1} and with a 14-h photoperiod (25°C by day and 18°C by night). The higher CO_2 concentration permitted all A52 progeny to grow but only a subset survived at ambient CO_2 (Mate et al., 1993). Plants were irrigated with a complete nutrient solution containing 8 mM nitrate every 2nd d and with water on other days.

Gas-Exchange Measurements and Experimental Protocol

Developmental Study with Plants Grown in High CO_2

We used a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, NE) to measure CO_2 assimilation rate, stomatal conductance, and intercellular partial pressure of CO_2 at an irradiance of 1000 μmol m^{-2} s^{-1}, a CO_2 partial pressure of 350 μbar, and a leaf temperature of 25°C. Plants were moved from the growth cabinet to the laboratory approximately 15 min before measurement, and data were recorded after the assimilation rate of the measured leaf segment had stabilized, approximately 30 min later. After gas-exchange measurements, leaf discs (0.517 cm^2 each) were removed under the same illumination from the same portion of the leaf on which the gas-exchange measurements had been made, snap-frozen in liquid N_2, and stored at −80°C.

Comparison of Youngest Mature Leaves of Plants Grown in High and Ambient CO_2

In these experiments we also measured extractable Rubisco activity, RuBP, and PGA. The sampling and measurement procedure was similar but the gas-exchange system was fitted with a freeze-clamping apparatus (Badger et al., 1984) and leaf discs were freeze-clamped in situ after the gas-exchange measurements. The freeze clamp was designed to produce a frozen leaf disc divided in half, with each half measuring 2.67 cm^2. One-half was used for measurements of Rubisco sites, Rubisco activity, soluble protein, and activase content, the other was used for PGA and RuBP assays. At the same time, another 0.517-cm^2 leaf disc was taken for measurement of chlorophyll content.

Rubisco Measurements

Rubisco catalytic site concentration and carbamylation status were determined by the stoichiometric binding of [^{14}C]CPBP as described by Butz and Sharkey (1989) and Mate et al. (1993).

Initial and total Rubisco activities were estimated by RuBP-dependent incorporation of $^{14}CO_2$ into acid-stable products by a modification of the method of Jiang et al. (1993). One-half of a freeze-clamped leaf disc (2.67 cm^2) was ground rapidly in a chilled glass homogenizer with 1 mL of ice-cold, CO_2-free extraction buffer containing 50 mM Hepes-NaOH buffer, pH 7.8, 20 mM $MgCl_2$, 10 mM DTT, 1 mM EDTA, 1 mM PMSF, and 1% (w/v) polyvinylpolypyrollidone. After centrifugation for 10 s at 4°C, 10 μL of the supernatant was added immediately to 164 μL of assay buffer (100 mM Hepps (*N*-2-hydroxyethylpiperazine-*N*′-3-propanesulfonic acid)-NaOH buffer, pH 8.3, and 20 mM $MgCl_2$) mixed with 20 μL of 5.1 mM RuBP and 6 μL of 0.5 M $NaH^{14}CO_3$ (approximately 2000 cpm $nmol^{-1}$) to measure initial Rubisco activity. After 60 s at 25°C, the reaction was terminated by the addition of 20 μL of formic acid. The samples were dried, and acid-stable ^{14}C was measured by liquid scintillation. To measure total Rubisco activity, 10 μL of extract was added to the same assay buffer mixture

lacking RuBP and incubated for 5 min at 25°C, and then RuBP was added to initiate the reaction.

In one experiment, powdered, frozen leaf material was added directly to carbamylation and activity assays to avoid any delay between extraction and assay. The 10th leaves of older, 18-leaf plants grown in 1500 μbar CO_2 were sampled 36 d after planting. Leaf discs were freeze-clamped under the growth conditions in the middle of the photoperiod. Just before extraction, the discs were ground to a fine powder in liquid N_2. To measure Rubisco carbamylation status, a sample of frozen leaf powder was added to a CO_2-free solution containing 50 mM Bicine-NaOH buffer, pH 7.8, 5 mM $MgCl_2$, 5 mM DTT, 1 mM EDTA, 0.2 mM PMSF, and 7 μM [carboxy-^{14}C]CPBP (99,000 cpm nmol^{-1}). After 30 min at 0°C, unlabeled CPBP was added to a final concentration of 1.5 mM to one aliquot, and $NaHCO_3$ and $MgCl_2$ were added to another aliquot to final concentrations of 14 and 19 mM, respectively. Both aliquots were then warmed to room temperature for 10 and 45 min, respectively, and centrifuged briefly, and protein-bound label in the supernatants was separated from unbound label by gel filtration at room temperature through 10-mL columns of Sephadex G-50 (fine) equilibrated with 20 mM Hepps-NaOH buffer, pH 8.0, containing 75 mM NaCl. The carbamylated Rubisco/total Rubisco ratio was determined from the ratio between the bound label in the two aliquots. To measure initial Rubisco activity, a sample of frozen leaf powder was added to a solution containing 44 mM Bicine-NaOH buffer, pH 8.3, 17 mM $MgCl_2$, 4 mM DTT, 1 mM EDTA, 0.2 mM PMSF, 15 mM $NaH^{14}CO_3$ (1700 cpm nmol^{-1}), and 0.5 mM RuBP at 25°C. At 30-s intervals formic acid was added to aliquots of the reaction mixture to 30% (v/v), unfixed label was removed by drying at 80°C, and the remainder was measured by scintillation counting. $^{14}CO_2$-fixing activity was expressed in terms of the total amount of Rubisco present in the assay. The latter was determined by adding [^{14}C]CPBP (99,000 cpm nmol^{-1}) to a final concentration of 7 μM to another aliquot of the reaction mixture. After 45 min at 25°C, protein-bound label in the supernatant of this aliquot was isolated by the gel-filtration procedure described above. $^{14}CO_2$ trapped in the Rubisco-Mg^{2+}-CO_2-CPBP complex contributed only insignificantly to the bound label because its specific radioactivity was only 1.7% of that of the CPBP. Fully activated Rubisco activity was determined using an identical procedure, except that the frozen leaf powder was added to the RuBP-free solution initially, and CO_2 fixation was initiated by adding RuBP 5 min after the leaf powder.

Activase Measurement

Activase content was measured by immunoblotting with enhanced chemiluminescence detection as described by Mate et al. (1996).

GAPDH Activity Assay

GAPDH activity was measured as described by Stitt et al. (1989). A 0.517-cm^2 leaf disc was extracted on ice in 200 μL of extraction buffer containing 50 mM Hepes-KOH, pH 7.4, 5 mM $MgCl_2$, 2 mM EDTA, 5 mM DTT, 2 mM benzamidine, 2 mM ε-amino-*n*-caproic acid, 0.1% (v/v) Triton X-100, and 1 mM PMSF. After centrifuging for 2 min, a 40-μL aliquot of the supernatant was added to a solution containing 100 mM Hepes-KOH, pH 8.0, 30 mM $MgCl_2$, 20 mM KCl, 2 mM EDTA, 4.5 mM DTT, 5.5 mM ATP, 0.2 mM NADPH, and 60 units of 3-phosphoglycerate kinase (Boehringer Mannheim), and incubated for 10 min at 25°C. The reaction, in a final volume of 1.1 mL, was started by the addition of 5.5 mM 3-phosphoglycerate and the rate was calculated from the initial rate of decline in NADPH A_{340}.

Metabolite Assays

RuBP and PGA were measured with a spectrophotometric, enzyme-linked assay. Each leaf disc half (2.67 cm^2) was ground in a glass homogenizer on ice with 200 μL of 5% (v/v) trifluoroacetic acid and centrifuged at 10,000*g* for 10 min. The supernatant was transferred to Eppendorf tubes and freeze-dried under vacuum. The residue was dissolved in 200 μL of distilled water, mixed with 2 mg of activated charcoal, kept on ice for 30 min, and centrifuged as before. The supernatant was used immediately for metabolite assays or snap-frozen in liquid N_2 and stored at −80°C. Purified spinach Rubisco was activated in 50 mM Hepps-NaOH buffer, pH 8.3, containing 20 mM $MgCl_2$, 20 mM $NaHCO_3$, and 1 mM EDTA, concentrated to 50 to 100 mg mL^{-1} by centrifuging in a concentrator (Centricon-30, Amicon, Beverly, MA), and preincubated for 10 min at 50°C in a stoppered Eppendorf tube. RuBP and PGA were measured consecutively in the same assay.

Leaf extract (50 μL) was added to an assay mixture (final volume of 2 mL) containing 90 mM Hepps-NaOH buffer, pH 8.0, 18 mM $MgCl_2$, 15 mM $NaHCO_3$, 0.2 mM NADH, 100 units of PGA kinase (Boehringer Mannheim), 40 units of glyceraldehyde 3-phosphate dehydrogenase (Boehringer Mannheim), and 100/35 units of a triosephosphate isomerase/glycerol-3-phosphate dehydrogenase mixture (Boehringer Mannheim). After the A_{340} became stable, PGA was measured from the decrement in absorbance following the addition of 5 μL of 1 M ATP. After the absorbance became stable again, RuBP was measured from the absorbance decrement following the further addition of 5 μL of activated, concentrated Rubisco solution. Trifluoroacetic acid extraction circumvents the inhibition of Rubisco by residual $KClO_4$ sometimes encountered when perchloric acid extraction is used.

Electron Microscopy

Small pieces (1.5 × 4 mm) of leaves were fixed in 0.1 M sodium cacodylate buffer containing 0.12 M Suc, 2.5% (v/v) glutaraldehyde, 3.5% (v/v) formaldehyde, 10 mM EDTA, and 2 mM $MgCl_2$ for 2 to 3 h. They were washed in the fixing solution without glutaraldehyde and formaldehyde and then postfixed in the same solution containing 0.5% (w/v) osmium tetroxide for 2 h. The samples were then dehydrated in a graded ethanol series and embedded in araldite. Sections were examined with a transmission elec-

tron microscope (model H600, Hitachi, San Jose, CA) at 75 kV.

Other Assays

Soluble protein was determined using the Coomassie Plus assay (Pierce) with BSA as standard. Chlorophyll was determined as described by Porra et al. (1989) after extraction of the leaf disc with 80% (v/v) acetone.

RESULTS

Developmental Studies with Plants Grown at High CO_2

Plant Growth

For the developmental studies, we used two sampling strategies (Fig. 1). In Set A, we followed one particular leaf during its lifetime. In Set B, we examined the youngest mature leaf just approaching full expansion near the top of the plant throughout the plant's lifetime. CO_2 enrichment, necessary to permit growth of all anti-activase plants, was used with all three genotypes. Plant height and leaf numbers were recorded from 2 weeks after planting until maximal height and leaf number were reached (Fig. 2). All three genotypes had similar growth profiles, attaining the same height and producing the same number of leaves. However, both transgenic lines grew more slowly than the wild type, with a 7-d delay in flowering and reaching final height and leaf number. The relationship between the height of a plant and the number of leaves it bore was the same regardless of genotype (Fig. 2E). This allowed a convenient way of circumventing genotypic differences in developmental age between plants of the same chronological age. These differences could be removed by expressing the developmental age of the plant in terms of the number of leaves it bore at the time of sampling.

Gas-Exchange Analysis

CO_2 assimilation rates were depressed to approximately 50% of wild-type rates in the anti-activase plants, and to 65% in the anti-GAPDH plants. These relative suppressions remained fairly constant throughout leaf and plant development (Fig. 3, A–C) despite all genotypes showing decreases in CO_2 assimilation rate as the leaves aged (Fig. 3, A and B). CO_2 assimilation rates of the youngest mature leaves of both wild-type and anti-activase plants did not change much as the plants aged (Fig. 3C). The partial pressure of CO_2 in the intercellular spaces during steady-state photosynthesis was higher in both types of transgenic plants than in the wild-type controls, so it is unlikely that the antisense-induced reductions in photosynthetic rate could be due to stomatal limitations. This parameter increased in all types of plants with leaf age (Fig. 3, D and E), but remained nearly constant in the youngest leaves of wild-type and anti-activase plants as the plants aged (Fig. 3F).

Biochemical Analyses

Young leaves of the anti-activase plants had less than 15% of the activase content of the wild-type plants, and activase declined to undetectable levels as the leaves aged (Fig. 3Q). The activase content of the youngest leaves of both wild-type and anti-activase plants was little affected by plant age, declining only moderately throughout plant development (Fig. 3R). GAPDH activity in anti-GAPDH plants was reduced to 5 to 10% of the wild-type activity

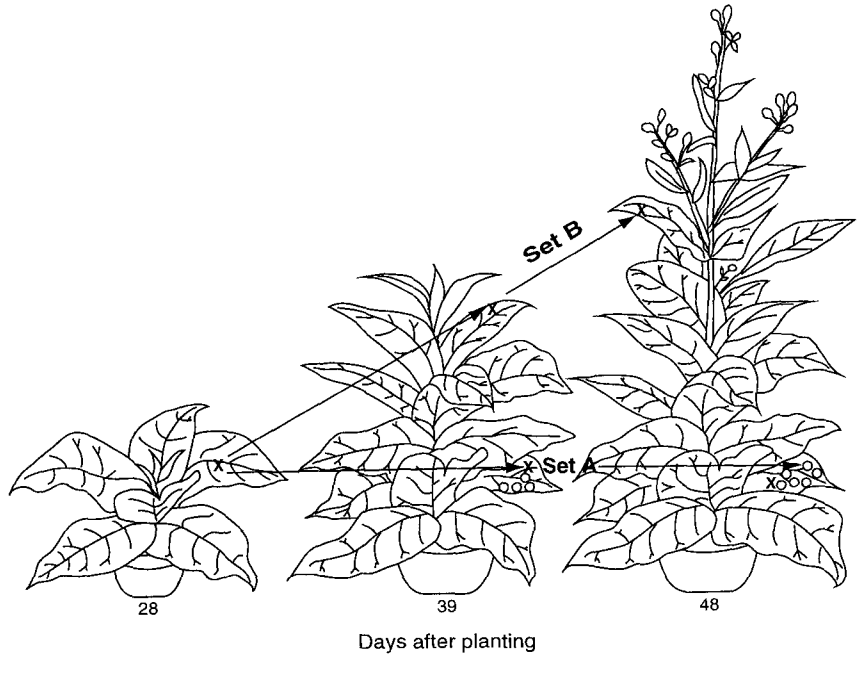

Figure 1. A schematic representation of tobacco plant and leaf development showing our sampling strategy for the developmental study at high CO_2. Leaves were numbered in order of emergence. Set A analyzes leaf development, repeatedly sampling one particular leaf throughout its lifespan. The ninth leaf was used for wild-type plants and the seventh for anti-activase and anti-GAPDH plants. Set B analyzes plant development, sampling only the youngest mature leaf near the top of each developing plant. The developmental age of a leaf or a plant was recorded in terms of the total number of leaves that it had at the time of sampling. The Set B sampling strategy was applied to wild-type and anti-activase genotypes only.

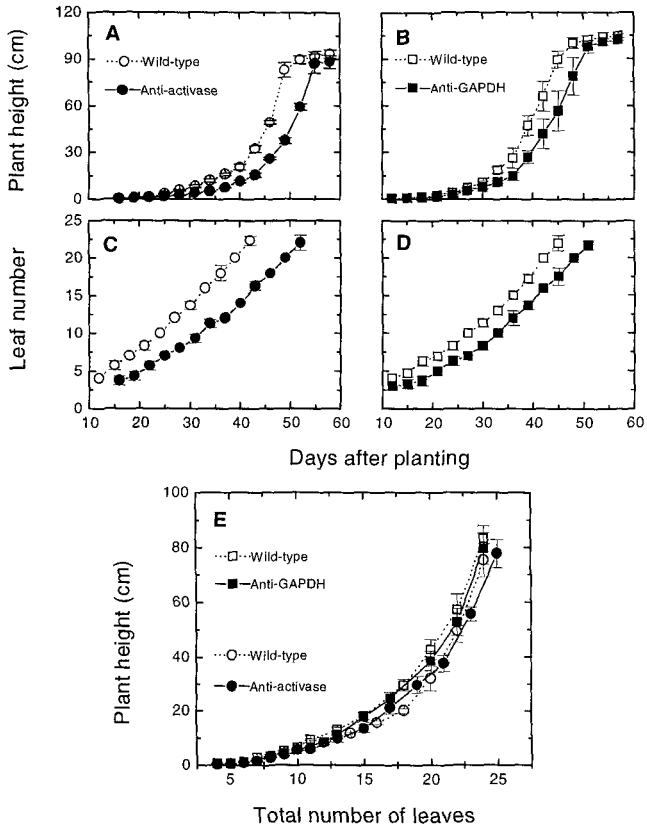

Figure 2. Growth of wild-type, anti-activase, and anti-GAPDH plants in 1500 μbar CO_2. Plant height (A and B) and leaf number (C and D) were recorded at 3-d intervals from 10 to 55 d after planting. Six wild-type plants for each control group, 15 anti-activase plants, and 15 anti-GAPDH plants were measured. The relationship between height and number of leaves was similar regardless of genotype (E). The means ± SE are plotted.

and it decreased with leaf age similarly in wild-type and anti-GAPDH plants (Fig. 3P). The contents of chlorophyll (Fig. 3, G–I) and soluble protein (Fig. 3, J–L) were nearly the same for all three genotypes. Both decreased with leaf age but remained fairly constant in young leaves as the plants aged.

Anti-activase plants retained more Rubisco in their older leaves than the control plants. The lower leaves on anti-activase plants with 14 leaves or more had approximately twice as much Rubisco as the comparable leaves on control plants (Fig. 3N). Anti-GAPDH plants had Rubisco contents similar to wild-type plants at early and late stages of leaf development, but higher contents at intermediate leaf ages (Fig. 3M). The increased Rubisco content induced by activase deficiency was not as apparent in the youngest mature leaves. Slightly more Rubisco was present early in the life of the plants, but, later, Rubisco content of the youngest leaves was similar to the controls (Fig. 3O).

The ratio of Rubisco to soluble protein changed drastically in anti-activase plants during leaf development (Fig. 4A). In young leaves on plants with fewer than eight leaves, the ratio was 0.26 for wild-type plants and 0.31 for anti-activase plants. In older leaves on plants with more than 18 leaves, the values changed to 0.10 and 0.30, respectively. The patterns of change were also different. The controls showed a progressive decline in the Rubisco/soluble-protein ratio as the leaves aged. In the anti-activase plants, the ratio first increased to reach the maximum (0.45) in middle age, and then declined (Fig. 4A). The larger Rubisco content and greater Rubisco/soluble ratio of older anti-activase leaves had little effect on the total content of soluble protein (Fig. 3K). Therefore, activase deficiency must cause reductions in the amounts of other soluble proteins to maintain Rubisco content. The Rubisco/soluble-protein ratio in the youngest mature leaves did not differ between wild-type and anti-activase plants, both showing a modest decline as the plants aged (Fig. 4B). The Rubisco/soluble-protein ratio was not affected by GAPDH deficiency nearly as much as by activase deficiency. In the anti-GAPDH plants, this ratio was mostly within the range seen in wild-type plants, rising a little higher than that seen in one set of controls only in middle-aged leaves (Fig. 4A).

Rubisco carbamylation was lower in the anti-activase plants than in the controls but the patterns of decline in carbamylation with leaf and plant age were similar (Fig. 4, C and D). The carbamylation percentages were 49% in young leaves and 32% in old leaves in anti-activase plants compared with the changes from 72 to 54% in wild-type plants (Fig. 4C). The youngest mature leaves of both wild-type and anti-activase plants showed smaller reductions in Rubisco carbamylation as the plants aged (Fig. 4D).

In the young leaves the ratio of activase monomers to Rubisco active sites was 0.1 to 0.2 throughout the life of the wild-type plants, but this ratio ranged from 0.01 to 0.02 for the anti-activase plants (Fig. 4F). However, the ratios changed in opposite directions as the leaves aged. The ratio decreased from 0.02 to less than 0.0025 in anti-activase plants at the same time as it increased from 0.15 to 1.0 in the controls (Fig. 4E).

Relationship between CO_2 Assimilation Rate and Carbamylated Rubisco Content

The ratio between the CO_2 assimilation rate and the content of carbamylated Rubisco reveals the rate of catalysis by carbamylated sites in vivo (Fig. 5). During leaf development, this ratio remained approximately constant in wild-type leaves but declined very markedly in anti-activase leaves to values approximately 10% of that seen in the wild type (Fig. 5A). These data confirm our previous observations with high-CO_2-grown, anti-activase plants (Andrews et al., 1995) and extend them by showing that leaf age has a critical influence on the phenomenon. The difference in Rubisco turnover rate in vivo between the two genotypes was also apparent, though much less pronounced, when only the youngest mature leaves were sampled. In the anti-activase plants, the turnover rate was always lower than in the wild type and tended to decline with plant age, whereas it tended to increase in the wild type (Fig. 5B). Our earlier studies revealed little reduction in the turnover rate of carbamylated Rubisco in youngest mature leaves of anti-activase plants grown without CO_2 supplementation (Mate et al., 1996).

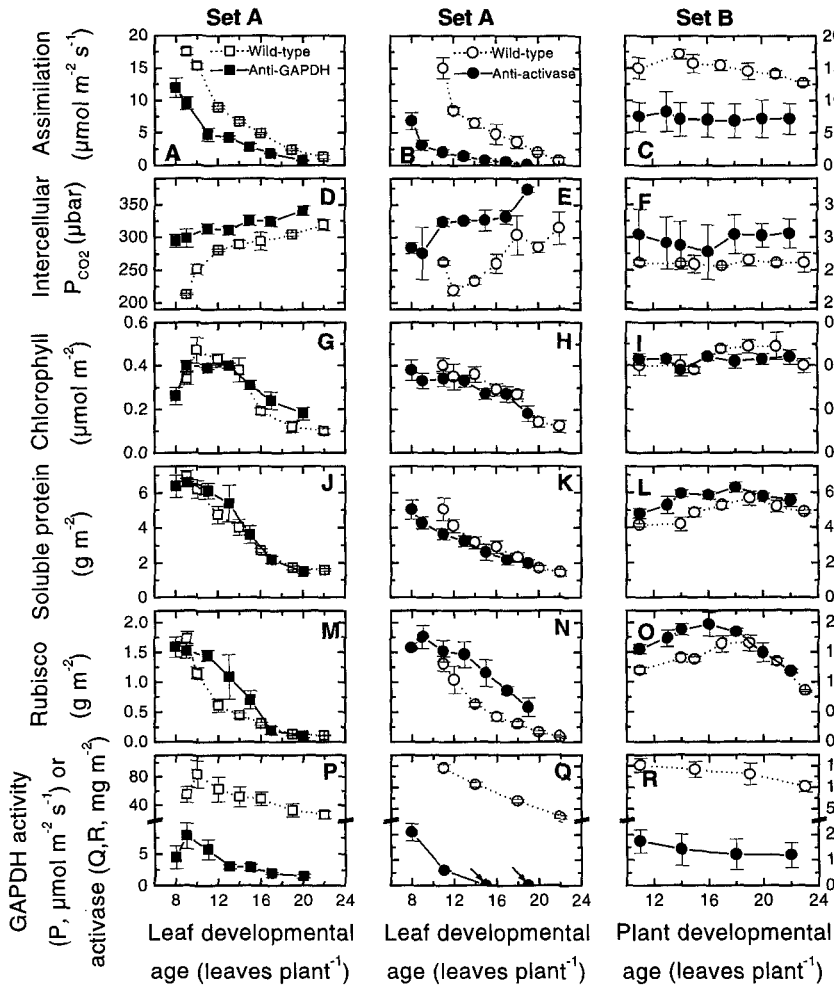

Figure 3. Gas-exchange and biochemical parameters of leaves of wild-type, anti-activase, and anti-GAPDH plants grown at 1500 μbar CO_2. The sampling strategies are outlined in Figure 1. Both leaf and plant age are expressed in terms of the total number of leaves that the plant had at sampling. For Set A, in which the same leaf was sampled throughout the life of the plant, the number of leaves the plant had at sampling quantifies the developmental age of the sampled leaf. For Set B, in which different leaves of a similar young age were sampled as the plant grew, the age of the plant is quantified as its number of leaves. The middle and right columns show the data for anti-activase plants and their wild-type controls during leaf development (Set A) and plant development (Set B), respectively. The left column displays the data for anti-GAPDH plants and controls during leaf development (Set A only). Data are presented as mean ± SE with $n = 3$. See "Materials and Methods" for experimental details. Measurements marked with arrows in Q revealed activase contents less than the detection limit of 2.6 mg m^{-2}. These data are plotted as zero.

Studies with the Youngest Mature Leaves of Plants Grown at Both High and Ambient CO_2

Biochemical Analyses

In another experiment we examined the relationship between Rubisco activity in vivo and in vitro after rapid extraction. The youngest mature leaves just approaching full expansion near the top of the plants were used. We grew 20 anti-activase and 10 wild-type plants at both high and ambient CO_2 concentrations. As in the previous experiment, the anti-activase plants grew well at high CO_2, although less rapidly than the wild type. Some anti-activase plants, however, could not maintain reasonable growth rates at ambient CO_2; these were discarded, and plants with a range of CO_2 assimilation rates were selected from those remaining. The wild-type plants grown at high CO_2 had somewhat lower activase contents (average 131 mg m^{-2}) than those grown at ambient CO_2 (average 215 mg m^{-2}). Anti-activase plants averaged 4 and 6% of the wild type's activase content at ambient and high CO_2, respectively, and their CO_2 assimilation rates were reduced over 75% (Table I). Some anti-activase individuals were much more repressed than the average, having activase contents as low as 1% of the wild type.

Wild-type leaves had similar amounts of chlorophyll, soluble protein, and Rubisco in both growth conditions, and CO_2 supplementation permitted the anti-activase plants to have similar contents. However, when grown at ambient CO_2, anti-activase plants were depleted in all of these parameters. Nevertheless, since Rubisco and soluble protein were similarly reduced in anti-activase plants grown at ambient CO_2, the ratio between them was not disturbed (Table I). The ratios between activase monomers and Rubisco active sites averaged 0.18 and 0.13 in wild-type plants grown at ambient and high CO_2, respectively. In anti-activase plants, these ratios decreased over 15-fold (Table I).

In accordance with previous observations (Mate et al., 1993, 1996), the wild-type plants grown at both CO_2 concentrations had similar Rubisco carbamylation levels (approximately 80%). Carbamylation was reduced in anti-activase plants, the reduction being somewhat greater for those grown in high CO_2 (average 42% carbamylated) than in those grown at ambient CO_2 (average 52% carbamylated) (Table I). Previous studies showed that anti-activase plants grown at ambient CO_2 had RuBP pool sizes similar to the wild type, but had reduced PGA pool sizes (Mate et al., 1996). In the present studies, we extended these mea-

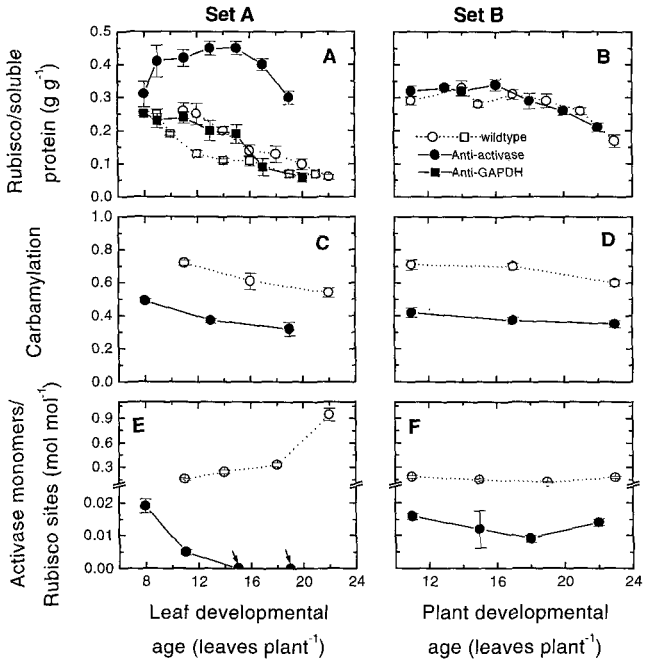

Figure 4. Rubisco carbamylation and relationships between Rubisco, soluble protein, and activase in wild-type and anti-activase plants grown in 1500 μbar CO_2. The Rubisco/soluble-protein ratios during leaf development in similarly grown anti-GAPDH plants are also shown in A. The data presented are calculated from those in Figure 3 and the method for expressing leaf and plant age is described in the legend of Figure 3. In E, ratios corresponding to the arrowed data points in Figure 3Q, where activase was undetectable, are plotted as zero. Based on this limit, the ratios must be less than 0.0025.

surements to high-CO_2-grown plants and again observed moderate suppression of PGA content in the anti-activase plants, to levels averaging 60% of those of the wild-type plants. However, anti-activase plants grown in high CO_2 had over twice as much RuBP as the controls (Table I).

Rubisco Activity in Vivo and in Vitro

The rate of catalytic turnover of Rubisco in vivo is given by the ratio between the CO_2 assimilation rate and the content of carbamylated Rubisco sites (Fig. 6, A and B). These data are in accordance with previous observations (Fig. 5; Andrews et al., 1995; Mate et al., 1996) and establish that the turnover rate of Rubisco in vivo is repressed by activase deficiency. The average Rubisco catalytic rate in vivo was reduced by 61 and 51% in anti-activase grown in high and ambient CO_2, respectively. Since the concentrations of both CO_2 and RuBP in the anti-activase leaves were similar to or greater than those prevailing in the wild type, and the PGA content was reduced by activase deficiency, slower turnover could not have been the result of substrate insufficiency or product accumulation.

The lowest turnover rates came from plants with the lowest activase levels and, in general, there appeared to be a hyperbolic correlation between the turnover rate of carbamylated Rubisco sites and activase content (Fig. 6, A and B). This suppression of turnover rate of carbamylated Rubisco in vivo was partially alleviated by extraction of the leaves. The average initial activity observed without preincubation immediately after leaf extraction was only 17 and 30% lower in the anti-activase leaves grown in high and ambient CO_2, respectively, compared with the wild type (Fig. 6, C and D). Similar, smaller reductions were seen when the total fully carbamylated activities were compared (Fig. 6, E and F). The nearly 3-fold greater turnover rates in vitro compared with in vivo are the result of the CO_2-saturating conditions prevailing in the in vitro assays.

Rubisco Activity in Vitro following Very Rapid Extraction of Older Leaves Grown in High CO_2

Recognizing that the possibility existed that the inactivation of Rubisco observed in anti-activase leaves might be the result of sequestration of Rubisco by a moderately tight-binding inhibitor that nevertheless managed to dissociate during the brief interval between extraction and assay inherent in the conventional procedure described in "Materials and Methods," we devised a procedure for measuring initial Rubisco activity that eliminated this delay. Frozen leaf discs were powdered in liquid N_2, and assays

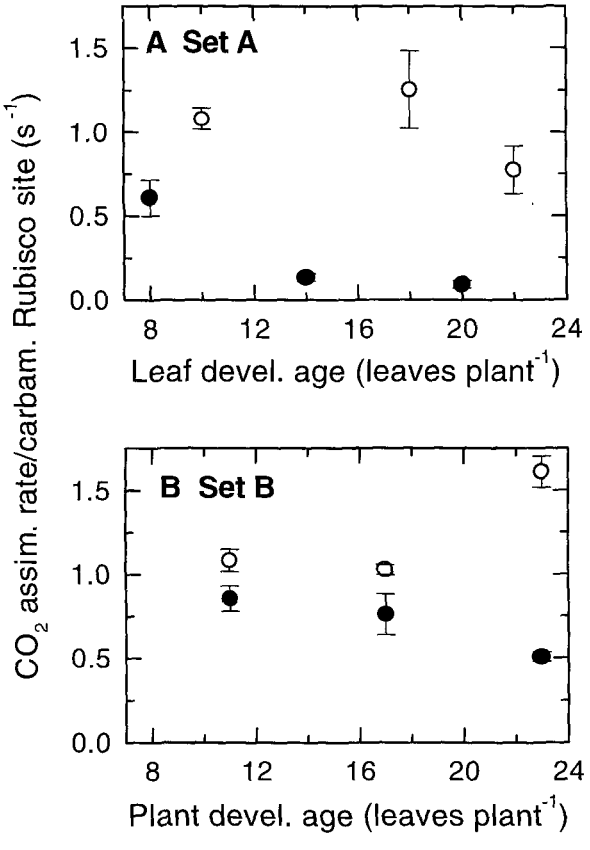

Figure 5. The catalytic rate of carbamylated Rubisco active sites in wild-type (○) and anti-activase (●) leaves during leaf (A) and plant (B) development in 1500 μbar CO_2. The Rubisco catalytic rate was calculated from measurements of CO_2 assimilation at 350 μbar CO_2. The method for expressing leaf and plant age is described in Figure 1 and in the legend of Figure 3. The data plotted are the means ± SE of measurements with three leaves.

Table I. *Influence of activase content on CO_2 assimilation rate and the contents of chlorophyll, soluble protein, Rubisco (total and carbamylated), RuBP, and PGA of the youngest mature leaves of wild-type and anti-activase plants grown in high (1500 μbar) and ambient (350–400 μbar) CO_2*

Values are the means ± SE with *n* shown in parentheses.

Parameter	High CO_2		Ambient CO_2	
	Wild type	Anti-activase	Wild type	Anti-activase
Activase (mg m^{-2})	131 ± 23 (3)	8.3 ± 3.9 (6)	215 ± 25 (3)	8.7 ± 5.8 (6)
CO_2 assimilation rate[a] (μmol m^{-2} s^{-1})	15.6 ± 2.3 (3)	3.7 ± 2.2 (6)	15.1 ± 1.9 (3)	3.5 ± 3.0 (6)
Chlorophyll (μmol m^{-2})	0.43 ± 0.04 (3)	0.44 ± 0.05 (6)	0.47 ± 0.06 (3)	0.26 ± 0.12 (6)
Soluble protein (g m^{-2})	4.9 ± 0.6 (3)	4.2 ± 0.2 (6)	4.9 ± 0.4 (3)	2.9 ± 1.0 (6)
Rubisco (μmol m^{-2})	23.0 ± 3.1 (3)	23.5 ± 2.2 (6)	26.3 ± 2.5 (3)	15.9 ± 5.4 (6)
Rubisco/soluble protein (g g^{-1})	0.32 ± 0.01 (3)	0.38 ± 0.03 (6)	0.37 ± 0.06 (3)	0.38 ± 0.05 (6)
Carbamylated Rubisco/total Rubisco	0.77 ± 0.07 (3)	0.42 ± 0.07 (6)	0.79 ± 0.01 (3)	0.52 ± 0.03 (6)
Activase monomers/Rubisco sites	0.129 ± 0.026 (3)	0.0075 ± 0.0028 (6)	0.184 ± 0.033 (3)	0.0112 ± 0.0045 (6)
RuBP[b] (μmol m^{-2})	122 ± 6 (4)	261 ± 29 (8)	110 ± 15 (4)[c]	130 ± 18 (9)[c]
PGA[b] (μmol m^{-2})	119 ± 12 (4)	73 ± 10 (8)	167 ± 34 (4)[c]	97 ± 32 (9)[c]

[a] Measured at 1000 μmol quanta m^{-2} s^{-1}, 350 μbar CO_2, and a leaf temperature of 25 °C. [b] Regardless of growth conditions, leaves were sampled by freeze-clamping after gas-exchange measurements at 350 μbar CO_2. [c] Data of Mate et al. (1996).

were started by adding samples of the powder directly to solutions containing all of the other ingredients. The results (Table II) were entirely consistent with those obtained with the conventional assay procedure (Fig. 6, C and E).

The turnover rate of carbamylated sites extracted from anti-activase leaves was only slightly less than that from wild-type leaves, a similar slight reduction also being seen when total activities were compared. No sign of acceleration that might reflect slow dissociation of an inhibitor was noticed in the time courses of these assays (not shown), nor was the ratio between the initial and total rates less than that predicted by the carbamylation ratio (Table II), as might be expected if an inhibitor were dissociating during the 5-min preincubation period required for measurement of total activity. Indeed, the initial/total activity ratio was slightly greater than the carbamylation ratio for both genotypes, which suggests that a small amount of inactivation might occur during the preincubation period, perhaps as a result of proteolysis.

Rubisco Aggregations Are Not Apparent in Anti-Activase Chloroplasts

We examined the chloroplasts of middle-aged anti-activase and wild-type leaves grown at high CO_2 by transmission electron microscopy. These leaves had large amounts of Rubisco and high ratios of Rubisco to soluble protein (Figs. 3 and 4). Growth at high CO_2 caused abundant accumulation of starch, particularly in the wild-type chloroplasts, but also, to a lesser degree, in the anti-activase chloroplasts. Otherwise, the anti-activase chloroplasts were morphologically similar to the wild type. Thylakoid membranes were well organized, and no crystalline or aggregated protein was visible (data not shown).

DISCUSSION

Growth of Wild-Type and Antisense Plants in High CO_2

Although atmospheric CO_2 enrichment allowed the survival of all anti-activase progeny, it did not fully restore their growth rate to that of the wild-type controls under the same conditions. Indeed, the residual growth impairment seen with the anti-activase plants even with CO_2 supplementation rivaled that of the anti-GAPDH plants (Fig. 2), in which the metabolic lesion would not be expected to be alleviated by high CO_2 (Price et al., 1995). Therefore, the physiological lesion induced by activase insufficiency must be only partially ameliorated by CO_2 enrichment, not fully repaired. Despite the reduction in growth rate, the antisense plants appeared quite normal. They attained the same height and number of leaves as the control (Fig. 2) and had similar amounts of chlorophyll and soluble leaf protein (Fig. 3).

Activase Deficiency Causes Rubisco to Persist Late into Leaf Development

Apart from the reduction in photosynthesis rate, the most obvious consequence of activase deficiency was the large increase in the ratio of Rubisco to soluble protein that occurred in older leaves (Fig. 4A). These data confirm our previous observations with anti-activase plants grown in high CO_2 (Mate et al., 1993; Andrews et al., 1995) and reveal the importance of leaf age as opposed to plant age. The present data show that young anti-activase leaves had little or no more Rubisco than control leaves (Figs. 3O and 4B; Table I), consistent with our previous observations with anti-activase plants grown in air (Mate et al., 1996). Therefore, the effect seems to be caused largely by a deferral of the normal decline in Rubisco content seen in control leaves as they develop (Fig. 3N). This persistence of Rubisco in older leaves may be a specific response to activase deficiency. Anti-GAPDH plants with similar photosynthetic impairments (Fig. 3A) had Rubisco contents and Rubisco/soluble protein ratios not much greater than those of the controls and the increase was confined to middle-aged leaves, with Rubisco decreasing with leaf age in a manner similar to the controls (Figs. 3M and 4A).

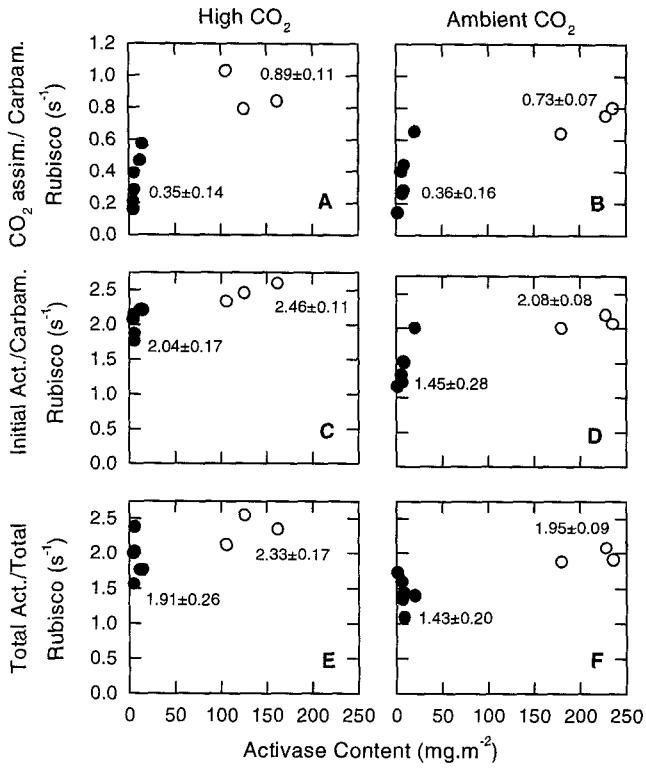

Figure 6. Influence of activase content of wild-type (○) and anti-activase plants (●) on the turnover rate of Rubisco in vivo (measured at 350 μbar CO_2) and in vitro (measured at CO_2 saturation). Data are shown for the youngest mature leaves of plants grown in 1500 μbar CO_2 (left column) and 350 to 400 μbar CO_2 (right column). Turnover rate in vivo is expressed as the ratio between the CO_2 assimilation rate measured by gas exchange and the content of carbamylated Rubisco sites (A and B). Turnover rate in vitro was measured after extraction of the leaves and is expressed in two ways: the initial rate immediately after extraction is expressed in terms of the content of carbamylated Rubisco sites (C and D) and the fully activated (or total) rate is expressed in terms of total Rubisco sites, both carbamylated and uncarbamylated (E and F). See "Materials and Methods" for further details.

The Activase/Rubisco Ratio Declines with Leaf Age in Anti-Activase Plants

Current models of activase function hypothesize encounters between ATP-primed activase and Rubisco that has a catalytically incompetent ligand bound to its active site. The encounter results in release of the ligand (Andrews et al., 1995; Portis, 1995; Mate et al., 1996; Salvucci and Ogren, 1996). RuBP bound to decarbamylated Rubisco is one example of such a catalytically incompetent complex; catalytic by-products bound to carbamylated or decarbamylated Rubisco are others. The stoichiometry between activase and Rubisco is therefore likely to have an important influence on the frequency of such encounters.

The anti-activase gene reduced the activase/Rubisco ratio approximately 10-fold in the youngest mature leaves, regardless of plant age (Fig. 4F). Like most photosynthetic proteins, the amounts of both Rubisco and activase decline as the wild-type leaf ages (Fig. 3, N and Q). However, Rubisco declined faster than activase, so that the activase/Rubisco ratio increased approximately 3-fold during leaf development in the wild type (Fig. 4E). By contrast, in the anti-activase leaves the persistence of Rubisco late into leaf development caused this ratio, which was already reduced to one-tenth of that of the wild type in the youngest mature leaves, to exhibit a further, substantial decline (Fig. 4E). By the time activase had declined below the limit of detection in the older leaves, a reduction in the activase/Rubisco ratio of several hundred-fold compared with the wild type had occurred.

How Is Rubisco Content Regulated?

Presumably, the persistence of large amounts of Rubisco late into leaf development is a response to the impairment of RuBP carboxylation induced by activase deficiency, but how is that impairment perceived? Expression of photosynthetic genes, including that of the Rubisco small subunit, is repressed by sugars (for review, see Sheen, 1994; Koch, 1996; Van Oosten and Besford, 1996), but is it likely that an undersupply of leaf carbohydrates is the cause for the delay in the decline in Rubisco content? If this was the case, we would expect to see a similar delay with the anti-GAPDH plants, which are also impaired photosynthetically.

The decline in Rubisco content was delayed much less in anti-GAPDH plants than in anti-activase plants (Fig. 4A). This argues against the persistence of Rubisco in anti-activase leaves being a general response to sugar deficit. However, it is possible that photosynthesis (and therefore carbohydrate supply) in the anti-GAPDH plants might be slightly less impaired than in the anti-activase plants, despite the similarity in developmental retardation (Fig. 2). Therefore, we must leave open the possibility that delayed redeployment of Rubisco might be a general consequence

Table II. *Turnover rate in vitro of Rubisco from older, high-CO_2-grown leaves following direct addition of powdered frozen leaf tissue to complete assay solutions*

Values are the means (± SE) of measurements with three leaves of each genotype. See "Materials and Methods" for experimental details.

Parameter	Wild Type	Anti-Activase
Carbamylated Rubisco/total Rubisco	0.75 ± 0.04	0.36 ± 0.03
Initial rate (mol CO_2 fixed mol^{-1} total Rubisco s^{-1})	2.19 ± 0.10	0.99 ± 0.03
Initial rate (mol CO_2 fixed mol^{-1} carbamylated Rubisco s^{-1})	2.93 ± 0.03	2.76 ± 0.31
Fully activated rate (mol CO_2 fixed mol^{-1} total Rubisco s^{-1})	2.54 ± 0.14	2.19 ± 0.06
Initial rate/fully activated rate	0.87 ± 0.08	0.45 ± 0.03

of lower sugar levels. This question needs to be addressed by a further study measuring leaf sugar content during leaf and plant development and comparing the anti-activase plants with other transgenic plants with, ideally, even greater photosynthetic impairments than the anti-GAPDH progeny displayed in the present study.

We consider that the present data point toward a more direct mechanism for sensing slowed RuBP carboxylation. Perhaps an imbalance in the pool sizes of phosphorylated intermediates during photosynthesis resulting from a carboxylation restriction can be perceived and signaled to the Rubisco synthesis and/or degradation machinery by currently unknown mechanisms. Although the leaf responds to the carboxylation impairment by delaying the remobilization of Rubisco, the strategy fails. With insufficient activase, the additional Rubisco retained is decarbamylated or otherwise inactivated (discussed below) and thus is not able to improve the rate of photosynthesis. As a result, the developmental progress of the leaf and the plant is retarded.

Lower Photosynthesis Rates Induced by Activase Deficiency Are Only Partially Explained by Reduced Rubisco Carbamylation

The carbamylation status of Rubisco was reduced moderately in the anti-activase leaves compared with the controls regardless of the CO_2 concentration during growth (Table I). This reduction was approximately constant throughout plant (Fig. 4D) and leaf (Fig. 4C) development. The modest reduction in carbamylation during leaf development (Fig. 4C) does not mirror the large decrease in activase/Rubisco ratio that occurred simultaneously (Fig. 4E). The activity of activase is known to be modulated by the concentrations of ATP and ADP (Portis, 1992), and it is possible that reductions in activase content could be partially compensated by increases in the ATP/ADP ratio induced by impaired RuBP carboxylation. Consequently, activase activity would not be reduced as much as activase content.

Although a reduction in carbamylation might be an expected consequence of reduced activase activity, in no case was the reduction sufficient to explain the reduced photosynthesis rates of the anti-activase plants. The CO_2 assimilation rate sustained in vivo at 350 μbar CO_2 by each carbamylated Rubisco site was lower in anti-activase plants than in wild-type plants regardless of the age of the plant or leaf or of the CO_2 concentration during growth (Figs. 5 and 6). As seen in preliminary experiments (Andrews et al., 1995), the reduction was particularly marked in leaves of plants grown in high CO_2, in which the activity of carbamylated sites decreased progressively as the anti-activase leaves developed. Eventually, in the oldest leaves the catalytic turnover rate of carbamylated Rubisco in anti-activase plants was only about one-tenth of that measured in the wild-type plants (Fig. 5A). Although this impairment was not obvious in our previous studies with young leaves of anti-activase plants grown in ambient CO_2 (Andrews et al., 1995; Mate et al., 1996), it was quite apparent with such leaves in the present experiment (Fig. 6B). The apparent hyperbolic response of the activity of carbamylated Rubisco to activase content (Fig. 6, A and B) is reminiscent of that of carbamylation itself (Mate et al., 1996). This similarity engenders suspicion that the two phenomena may be related mechanistically.

Possible Causes for Slower Turnover of Carbamylated Rubisco Induced by Activase Deficiency

Slower turnover of carbamylated Rubisco sites was not a result of substrate limitation or of product inhibition. RuBP content was increased by activase deficiency, particularly when the plants were grown in high CO_2, whereas PGA content was reduced (Table I). Therefore, the impairment seems to be intrinsic to Rubisco itself. Possible explanations for the impairment fall into two classes, categorized according to whether an indirect or a direct role for activase is hypothesized.

The indirect class hypothesizes that some feature of the stromal environment affecting Rubisco activity is altered by activase deficiency. Perhaps disruption of the regulation of Rubisco and the consequent imbalance in the concentrations of RuBP and PGA lead to stromal alkalization, which reduces Rubisco activity. This seems unlikely, however, because similar reductions of Rubisco activity and increases in RuBP/PGA ratio are observed in anti-Rubisco plants (Quick et al., 1991; Mate et al., 1996), but no signs of inhibition of carbamylated Rubisco were observed (Andrews et al., 1995). Another possibility might be some form of physical barrier to substrate (particularly RuBP) access when large amounts of Rubisco are present, as is the case in anti-activase plants grown in high CO_2. Although we could detect no ultrastructural sign of such a barrier, such as protein aggregates or crystals, less visible physical barriers are conceivable.

The direct class is based on current ideas about the mechanism by which activase modulates the activity of Rubisco (Portis, 1992; Mate et al., 1996; Salvucci and Ogren, 1996). Powered by ATP hydrolysis, activase is envisaged to promote dissociation of ligands bound unproductively to Rubisco. This might be achieved by activase binding specifically to the form of Rubisco that has several mobile loops closed over the ligand at the active site and coupling energy derived from ATP hydrolysis to induce retraction of these loops, opening the active site and allowing release of the ligand (Andrews et al., 1995; Andrews, 1996). Examples of unproductive ligand binding include the substrate RuBP bound to the uncarbamylated site, and nonreactive substrate analogs such as CA1P bound to the carbamylated, metal-occupied site. Binding of an inhibitor to carbamylated sites might be expected if insufficient activase were present to ensure its release. This would provide a ready explanation for our observation that carbamylated sites turn over more slowly, on average, in anti-activase leaves. Such an inhibitor would be expected to remain bound during the rapid extraction and assay procedures. For example, the presence of CA1P reduces both initial and total Rubisco activities in leaf extracts (Kobza and Seemann, 1989), being displaced only by high salt concentrations or more tightly binding ligands, such as 2′-carboxyarabinitol-

1,5-bisphosphate. Other ligands that bind as tightly as CA1P would be expected to behave similarly.

Our in vitro data indicate that this kind of tight-binding inhibitor might have a role in explaining the impairment of carbamylated Rubisco, but only a minor one, because most of the impairment vanished upon extraction of the leaves. Initial Rubisco activity per carbamylated site in the extracts of anti-activase leaves was, indeed, less than that measured in wild-type extracts, but only slightly so, particularly in high-CO_2-grown leaves (Fig. 6, C and D). This remained true even when powdered, frozen leaves were directly added to the assay solution to eliminate any delay between extraction and assay (Table II). Therefore, inhibition by a tight-binding inhibitor that is normally released by activase must be relegated to only a minor role in causing the slower turnover.

Parry et al. (1997) recently detected small amounts of an unidentified inhibitor with these binding characteristics in tobacco leaves during the photoperiod. When the plants were grown in ambient CO_2, the difference in initial activity of carbamylated Rubisco between anti-activase and wild-type leaves was more significant (Fig. 6D). These anti-activase plants have considerably lower Rubisco levels (Table I), which might make the effects of even small amounts of an inhibitor more apparent. Nevertheless, even in this case, much of the catalytic impairment of carbamylated sites in vivo did not survive extraction. In all cases, the reduction in initial activity per carbamylated site in anti-activase leaves was mirrored by a similar reduction in the total activity per total sites (Fig. 6, E and F; Table II). If this latter reduction is caused by the same inhibitor, the inhibitor must be unselective, binding to carbamylated and uncarbamylated sites equally well.

Catalytic inhibition in vivo by a loosely binding inhibitor that dissociated immediately upon the dilution inherent in extraction of the leaves would be consistent with the inactivation seen in vivo but not in vitro. However, a very rapidly released inhibitor would have no need of activase to assist its release and would thus not be expected to exert its influence specifically when activase was lacking.

Another possibility falls within the direct class of potential explanations but would require a radical extension of current concepts about activase function. Activase might also function to correct some kind of conformational error that Rubisco becomes susceptible to in the stromal environment, perhaps potentiated by the extremely high concentrations of Rubisco that prevail in vivo. Such a problem might be rapidly ameliorated by the dilution associated with extraction. This postulated role would have some elements akin to the role of chaperone proteins; Sánchez de Jiménez et al. (1995) have suggested that activase belongs to the chaperone group. Molecular chaperones are a group of proteins, often with ATPase activity, that assist in the folding and assembly of other proteins. Some are considered also to assist in correcting the misfolding of proteins that occurs during heat shock or other stresses (Ellis, 1993). However, noting that activase was unable to restore the activity of heat-denatured Rubisco, Eckhardt and Portis (1997) doubted that categorization of activase as a chaperone was warranted. Unfortunately, a chaperone-like activity that corrected a specific conformational problem that was manifested only in the highly concentrated stromal environment would not be readily amenable to study in vitro.

Krapp and Stitt (1995) studied the inhibition of photosynthesis induced in spinach leaves following application of a cold girdle to the petiole. They observed that the inhibition could not be explained in terms of a reduction in the initial activity of Rubisco after rapid extraction or in terms of RuBP scarcity or PGA accumulation. Indeed, in their experiments, Rubisco activity in vivo appeared to decrease at a time when both the RuBP/PGA ratio and the carbamylation status of Rubisco were increasing. They suggested that the activity of carbamylated Rubisco sites was being reduced by an unknown mechanism in response to carbohydrate accumulation induced by cold girdling. Grub and Mächler (1990) also reported a reduction in the catalytic effectiveness of activated Rubisco while carbohydrates were accumulating following transfer of low-light-grown red clover to a higher light intensity. The similarity between our present observations and those of Krapp and Stitt (1995) and Grub and Mächler (1990) encourages speculation that there might be a common underlying mechanism. Perhaps in some unknown way, carbohydrate accumulation induces by another means the same reduction in the ability of activase to stimulate catalysis by carbamylated Rubisco that we observe as a result of reducing the content of activase itself.

In summary, these studies establish that, in vivo, activase influences the rate of catalytic turnover by carbamylated Rubisco as well the carbamylation status of Rubisco. The mechanism by which activase effects this modulation of the Rubisco turnover rate will be an interesting topic for further study.

ACKNOWLEDGMENT

We thank Heather Kane for helpful discussions, practical assistance, and for reading the manuscript.

Received July 16, 1997; accepted September 9, 1997.

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