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Evidence for Lack of Turnover of Ribulose 1,5-Diphosphate Carboxylase in Barley Leaves

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ABSTRACT

Turnover of ribulose 1,5-diphosphate carboxylase in barley leaves (*Hordeum vulgare* L.) was followed over time in light and dark. The enzyme was degraded in prolonged darkness and was resynthesized after the plants were returned to light. Labeling with ^{14}C showed that simultaneous synthesis and degradation (turnover) did not occur in light. In contrast, the remaining soluble protein was turned over rapidly in light. Although ribulose 1,5-diP carboxylase can be both degraded and synthesized, these processes seem not to occur simultaneously, but can be induced independently by changing environmental conditions.

Much information is available on the increase of RuDP[1] carboxylase activity (6, 3, 9, 12, 13, 16), RuDP carboxylase protein (9), and fraction I protein (9, 10), in response to leaf development and light, and relatively little on the fate of the enzyme after its synthesis. Fraction I protein and RuDP carboxylase are very probably identical (9, 14, 15, 18, 19). RuDP carboxylase activity and fraction I protein decrease during senescence of tobacco (1, 8) and Perilla (7) leaves, but it is not known whether the enzyme is controlled by a turnover system or is degraded without simultaneous synthesis.

Since RuDP carboxylase protein makes up a large percentage of the total soluble protein of many plant leaves, it can be considered to be a major component of storage proteins (9, 14) as well as a major catalyst of CO_2 fixation. A study was therefore done to determine whether RuDP carboxylase is controlled by a turnover system in barley leaves.

MATERIALS AND METHODS

Plant Materials. *Hordeum vulgare* L. var. Numar was grown in vermiculite in 24-cm plastic pots or 28- × 33-cm plastic pans. Moisture was supplied by cotton wicks connecting the vermiculite to a full strength nutrient supply (9). The seedlings were grown for 6 days in either complete darkness or in continuous light (21,000 lux) at 27 C and 55% relative humidity.

Plant Treatments. Loss of RuDP carboxylase from barley seedlings was followed in both darkness and continuous light. Six-day-old light-grown seedlings were placed in darkness, and the level of the enzyme was assayed every 6 hr for 72 hr. The seedlings were then returned to light, where the enzymatic assays were continued.

This report defines turnover as simultaneous synthesis and degradation of protein. To determine whether RuDP carboxylase was being turned over while plants were in continuous light, RuDP carboxylase protein was labeled with ^{14}C by introducing $^{14}CO_2$ when the leaves were rapidly synthesizing the enzyme (6, 9, 13). Six-day-old dark-grown barley seedlings were given 12 hr of light and then placed in a gas-tight chamber at 27 C under light (16,000 lux) and treated with 2 mc of $^{14}CO_2$. Continuous monitoring showed that at the end of 6 hr, about 80% of the $^{14}CO_2$ had been removed from the atmosphere of the chamber by the seedlings. At that point the plants were removed from the chamber and placed in continuous light (21,000 lux) at 27 C and 55% relative humidity. Assays were begun after a 24-hr equilibration period.

Preparation of Cell-free Extracts. The top 10 cm of the first leaf of 10 seedlings were excised and ground with mortar and pestle in 0.1 M tris-SO_4 buffer, pH 8.0 (3 ml/g leaf tissue). The homogenate was centrifuged for 15 min at 30,000*g*, and the supernatant was used for the enzyme assay, for soluble-protein determination, and for reaction with a specific antibody (9).

Carboxylase Assay. RuDP carboxylase activity in the cell-free extracts was determined by the method of Kleinkopf *et al.* (9). All assays were run in complete darkness at 28 C.

Protein Determinations. The soluble protein of the cell-free extracts was precipitated with trichloroacetic acid (final concentration of trichloroacetic acid 5%) and assayed by the method of Lowry *et al.* (11).

Antigen-Antibody Precipitation. The concentration of RuDP carboxylase in each cell-free extract was determined by precipitation with a RuDP carboxylase-specific rabbit antibody (9). The antibody-antigen precipitate was solubilized with 0.2 ml of 0.05 M NaOH, and the amount of protein present was determined by the method of Lowry *et al.* (11). The ^{14}C incorporated into RuDP carboxylase was measured by dissolving the antibody-antigen precipitate in 0.05 M NH_4OH, drying the solution on filter paper, and counting with a liquid scintillation counter.

Specific Activity. The term "specific activity" is used in two ways herein. First, the specific activity of the enzyme RuDP carboxylase was determined as enzymatic activity per mg of RuDP carboxylase protein, and is called "enzymatic specific activity." Second, the specific activity of the radioactive carbon fixed in the plant was determined as either radioactivity per mg of RuDP carboxylase protein or radioactivity per mg of soluble protein minus RuDP carboxylase protein and is called "specific radioactivity."

RESULTS AND DISCUSSION

To determine the stability of the enzyme, RuDP carboxylase protein and activity were followed in the first leaf of barley growing in both light and dark (Fig. 1). Plants kept in continu-

[1] Abbreviation: RuDP: ribulose 1,5-diphosphate.

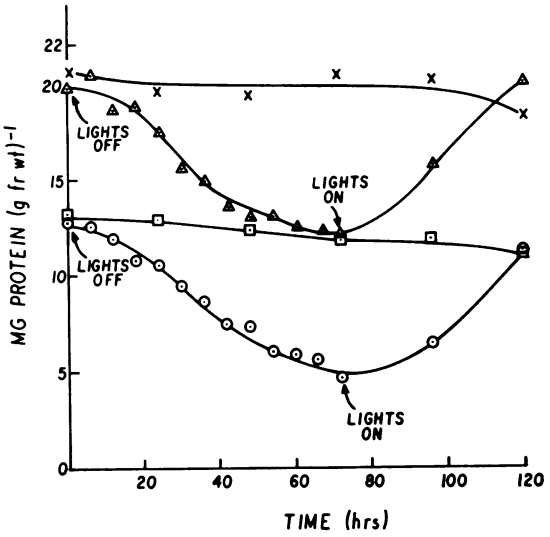

FIG. 1. Effect of light and dark periods on leaf contents of total soluble protein and RuDP carboxylase protein. Barley seedlings were grown in light for 6 days, after which light and dark treatments were begun. ×, △: Total soluble protein; ⊡, ⊙: RuDP carboxylase protein; ×, ⊡: continuous light; △, ⊙: 72 hr of darkness, then 48 hr of light. $LSD_{0.05,\ 0.01}$ (×) 3.0, 4.1; (△) 2.8, 3.8; (⊡) 2.9, 4.0; (⊙) 1.0, 2.6.

ous light maintained RuDP carboxylase protein and soluble protein for about 120 hr (seedlings now 11 days old) with very little loss (Fig. 1).

Since the leaves did not lose a significant amount of their RuDP carboxylase protein until after 24 hr of darkness, the plants were allowed to remain longer in darkness to determine losses during longer periods. Leaf content of RuDP carboxylase protein continued decreasing until only 40% remained at 72 hr (Fig. 1). RuDP carboxylase protein decreased by about 8 mg (g fr wt)$^{-1}$, and total soluble protein by approximately 9 mg (g fr wt)$^{-1}$, during the 3-day dark period, showing that 90% of the loss of soluble protein was due to the disappearance of RuDP carboxylase. During the ensuing light period, the seedlings regained all of the carboxylase protein and soluble protein lost during the dark period. The increase in RuDP carboxylase protein when the plants were returned to light accounted for about 90% of the increase in soluble protein.

Loss of RuDP carboxylase activity was not significant until after 30 hr of darkness (Fig. 2). All of the activity lost was regained during the ensuing light period. The enzymatic specific activity of RuDP carboxylase increased during the dark period and, again, decreased to that of the light control when the plants were returned to light (Fig. 3). The enzymatic specific activity of plants maintained in light remained constant during the 5-day experimental period.

Changes in the enzymatic specific activity of RuDP carboxylase have been reported earlier. Kawashima and Mitake (8) found that the activity of the carboxylase decreased more rapidly than did fraction I protein as tobacco leaves aged. As mentioned above, fraction I may be considered as crude RuDP carboxylase. W. R. Anderson and L. W. Peterson (personal communication, Brigham Young University) have evidence that the specific activity of the carboxylase can be changed by manipulations of temperature or day length or both. Changes in specific activity could also be related to effects on the light-activating factor which may be attached to RuDP carboxylase as reported by Wildner and Criddle (20).

The above results show that RuDP carboxylase protein is maintained at a relatively constant level under conditions of active photosynthesis. After being placed in darkness the leaves lost RuDP carboxylase protein, indicating the presence of a degrading system. The increase in RuDP carboxylase protein after the plants were returned from darkness to light shows that a synthetic capability was still present.

To determine whether RuDP carboxylase was being turned over while its concentration remains quite constant, *i.e.*, in light (Fig. 1), the protein was labeled with ^{14}C during its light-induced synthesis. Radioactivity in the protein-free supernatant fraction decreased rapidly and reached a steady state after 24 hr (Fig. 4). Loss of radioactivity in soluble protein (minus RuDP carboxylase) (Fig. 4B) was greatest between 12 and 48 hr. In contrast, the radioactivity in RuDP carboxylase protein remained constant for about 72 hr and then declined slightly. Carboxylase protein also remained constant for about 72 hr and then showed a slight loss (Fig. 5). Although the ^{14}C content of the remainder of the soluble protein was decreasing rapidly (Fig. 4), its concentration remained constant for about 60 hr and then declined slightly (Fig. 5).

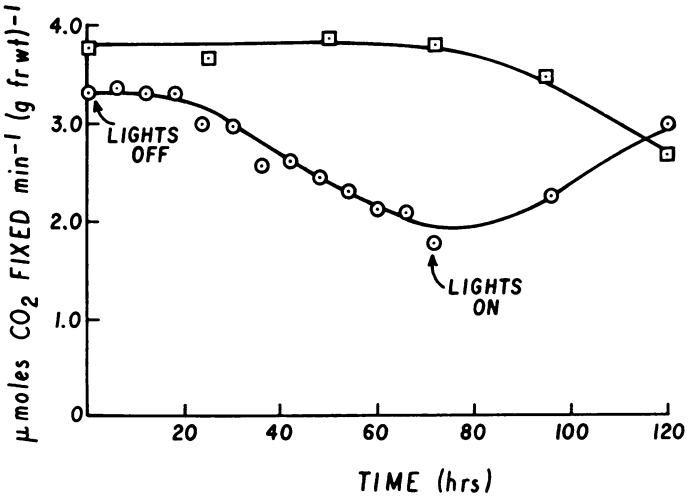

FIG. 2. Effect of light or dark periods on RuDP carboxylase activity. Activities were from treatments in Fig. 1. ⊡: Continuous light $LSD_{0.05,\ 0.01}$ 0.69, 0.95; ⊙: 72 hr darkness then 48 hr of light $LSD_{0.05,\ 0.01}$ 0.63, 0.84.

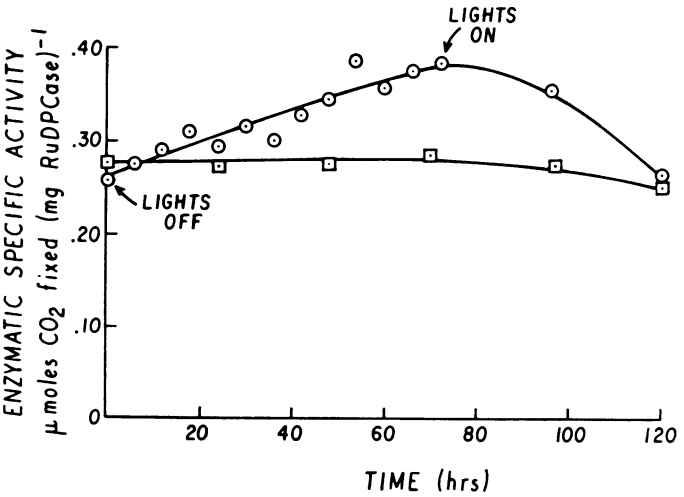

FIG. 3. Effect of light and dark periods on enzymatic specific activity. See Fig. 1 for treatments. ⊡: Continuous light $LSD_{0.05,\ 0.01}$ 0.063, 0.084; ⊙: 72 hr of darkness, then 48 hr of light $LSD_{0.05,\ 0.01}$ 0.039, 0.052.

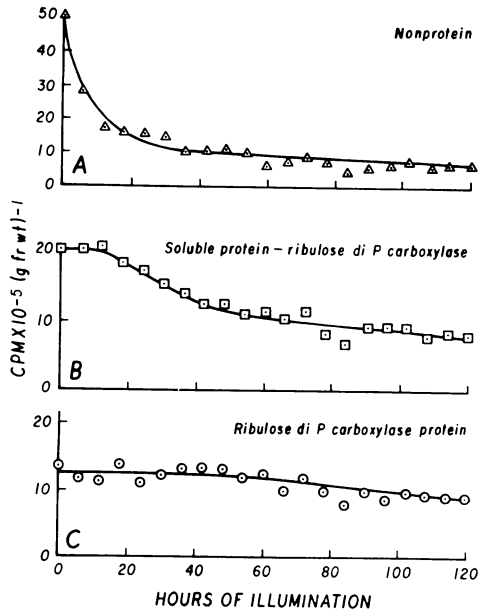

FIG. 4. Time course curves for ^{14}C content of soluble fractions of barley leaves. Six-day-old dark-grown barley seedlings were given 12 hr of light, then treated with $^{14}CO_2$ for 6 hr. Assays were begun after a 24-hr equilibration period in light and $^{12}CO_2$. See "Materials and Methods" for further details. A: Nonprotein fraction (^{14}C left in the cell-free extract after all trichloracetic acid-precipitable protein had been removed), $LSD_{0.05,\ 0.01}$ 4.0, 5.36; B: soluble protein minus RuDP carboxylase, $LSD_{0.05,\ 0.01}$ 6.6, 8.9; C: RuDP carboxylase, $LSD_{0.05,\ 0.01}$ 1.81, 2.5.

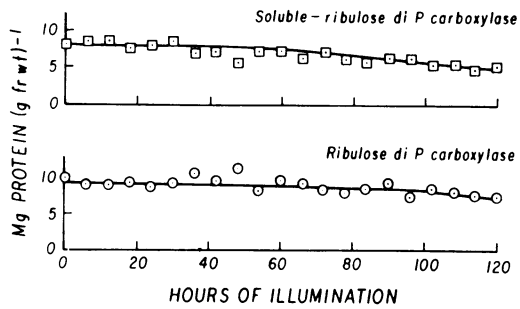

FIG. 5. Time course curves for the leaf concentration of soluble protein minus RuDP carboxylase protein (⊡) $LSD_{0.05,\ 0.01}$ 1.9, 2.7, and RuDP carboxylase protein (⊙) $LSD_{0.05,\ 0.01}$ 1.4, 1.9. See Fig. 4 for treatments.

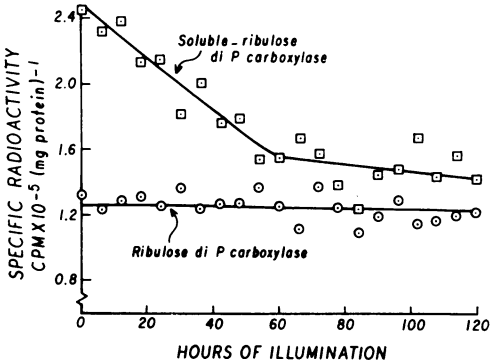

FIG. 6. Time course curves of specific radioactivity of soluble protein minus RuDP carboxylase protein (⊡) $LSD_{0.05,\ 0.01}$ 0.72, 0.96 and of RuDP carboxylase protein (⊙) $LSD_{0.05,\ 0.01}$ 0.34, 0.45. See Fig. 4 for treatments.

The specific radioactivity of RuDP carboxylase remained constant during the experiment (Fig. 6). A constant specific activity indicates no turnover (simultaneous synthesis and degradation) of RuDP carboxylase during the experiment. If turnover had occurred, the label would be diluted and the specific radioactivity would decrease. Dilution of label did occur with other soluble proteins (Fig. 6). The specific radioactivity of other soluble proteins decreased significantly during the first 50 hr of the experiment, showing that protein turnover did occur.

The rapid turnover of soluble protein other than RuDP carboxylase occurred at a time when the plants were essentially completing their adaptation to the shift from dark to light (6, 9). Dark-grown plants adjust to light in manifold ways. Chlorophyll is synthesized (6, 10, 12, 13), noncyclic photophosphorylation systems are developed (4), chloroplast development is completed (2), photosynthetic CO_2 fixation is increased (17), and activity of photosynthetic enzymes increases (6, 9, 13). In barley leaves, several of these processes proceed very rapidly during the first 24 hrs of light and reach a steady state sometime after about 36 to 48 hr of light (6). Hellebust and Bidwell (5) observed that turnover of soluble protein in wheat leaves was fastest when the leaves were either growing or biochemically differentiating. After growth or biochemical differentiation was completed, the turnover was very slow. Growth was not a confounding factor in our work, since the experiments were begun only after the first leaf had completed its growth (6). One possible interpretation of our results is that the major part of the turnover of soluble protein other than RuDP carboxylase occurred during the latter part of the biochemical adaptation to light conditions. Turnover was then very slow during the last 70 hr of the experiment, after adaptation was more nearly completed.

The concentration of RuDP carboxylase protein in the first leaf of barley does not appear to be under the constant control of a turnover system, simultaneous synthesis, and degradation. The protein is degraded during prolonged periods of darkness and can again be synthesized after plants are returned to light. No evidence was found, however, for simultaneous synthesis and breakdown during a light period. Although the enzyme can be both degraded and synthesized, these processes seem not to occur simultaneously but are rather induced independently by changing environmental conditions. In contrast, the remaining soluble protein showed rapid turnover in light during the first 50 hr of the experiment.

RuDP carboxylase was remarkably stable after its synthesis. Losses were not significant until after 24 hr of darkness (Fig. 1). Since RuDP carboxylase protein comprises a large percentage of the total soluble protein of many plant leaves it has been defined as a major storage protein (9, 14). Its stability after synthesis fits well with such a definition.

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