Plant Physiol. (1970) 45, 579–582

Influence of Age and Illumination on Distribution of Several Calvin Cycle Enzymes in Greening Barley Leaves

Received for publication October 31, 1969

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ABSTRACT

Activities of phosphoriboisomerase, phosphoribulokinase, and ribulose 1,5-diphosphate carboxylase, protein content, and chlorophyll accumulation in dark-grown barley seedlings were measured before and after illumination. Enzymatic activities, levels of soluble protein, and accumulation (upon illumination) of chlorophyll in leaves declined from tips toward the base. In response to increasing time of illumination, chlorophyll accumulation and activities of phosphoribulokinase and ribulose 1,5-diphosphate carboxylase (enzymes located in chloroplasts) increased most in tip portions whereas activity of phosphoriboisomerase and levels of soluble protein (constituents not confined to chloroplasts) increased similarly in all sections of the leaf. Maximum activity of phosphoribulokinase and maximum accumulation of chlorophyll shifted toward median portions of the leaf blade with increased age of seedling before illumination. Maximum activity of ribulose 1,5-diphosphate carboxylase and maximum level of soluble protein occurred in all leaf sections when the seedlings were 7 days of age before illumination.

Upon the illumination of dark-grown seedlings, chlorophyll accumulates, protein is synthesized, chloroplasts develop an ordered lamellar form, the activity of photosynthetic enzymes increases, and net incorporation of CO_2 begins. Some information is available on the development of several of these and other components with position in the leaf and age of the leaf. Rhodes and Yemm (13) reported that chloroplasts were larger and developed faster in the older cells of barley leaf tips than in younger cells at the base. Protochlorophyll levels in etiolated barley leaves declined from the tip toward the base (17). A decline in the tip with increasing age, however, shifted the maximal concentration toward the median sections. Williams and Rijven (19) noted marked differences in the timing of maximum protein and RNA levels for different parts of developing wheat leaves. Information is lacking on the development of photosynthetic enzymes in relation to position in the leaf and the development of other constituents. This paper reports the influence of illumination and leaf age and section on the activities of phosphoriboisomerase, phosphoribulokinase, and ribulose-1,5-diP carboxylase, and on soluble protein and chlorophyll.

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MATERIALS AND METHODS

Plant Materials. Etiolated barley plants (*Hordeum vulgare* L. var. Club Mariout) were produced by sowing seeds in vermiculite in plastic pots placed on top of pint jars filled with half-strength nutrient solution. A cotton wick connected the vermiculite in the pot with the solution in the jar and supplied the seedlings with sufficient moisture during the test periods.Prior to light treatment the plants were grown for 7 days in aerated light-proof boxes in a growth chamber at 24 C (Figs. 1–6).

Light treatments, nutrient solutions, and preparation of ribulose-1,5-diP were as previously reported (7). Phosphoriboisomerase, phosphoribulokinase, and ribulose-1,5-diP carboxylase were assayed as reported previously (7) except that 10 μmoles of $KH^{14}CO_3$ with a specific radioactivity of 2×10^5 cpm/μmole were used in the assay for ribulose-1,5-diP carboxylase activity. The activity of each enzyme refers to that determined from a cell-free extract.

Preparation of Cell-free Extracts. First-leaf blades of 50 seedlings were harvested and sectioned at 2-cm intervals from the tip; ground with mortar and pestle in 3 ml of 0.2 M tris buffer, pH 8.0, per g of leaf tissues; and centrifuged for 15 min at 37,000g. The supernatant liquid was diluted appropriately for each assay (7) and used as the source of enzymes and for analysis of extractable protein. After harvest all preparatory procedures were carried out at 0 to 3 C.

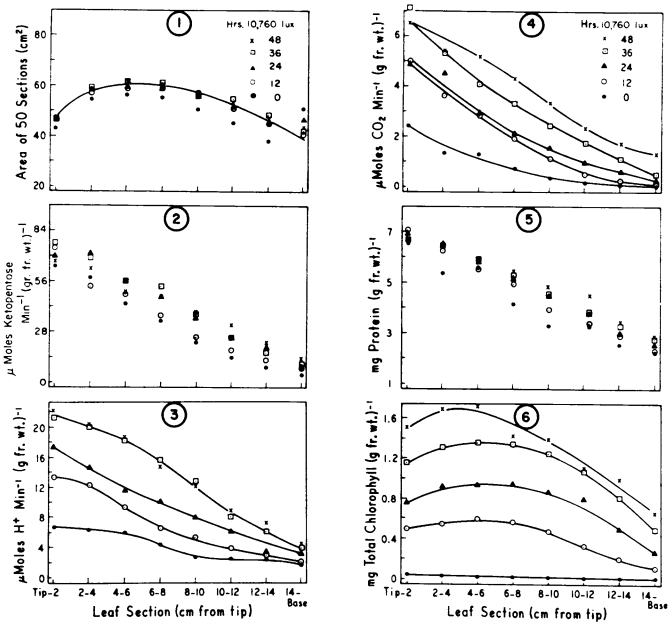
Soluble protein was determined by the method of Lowry *et al.* (10). The standard was tyrosine (1 mg tyrosine = 16 mg of protein). Chlorophyll was determined spectrophotometrically from 80% (v/v) acetone extracts according to Arnon's (1) modification of the method of MacKinney (12).

RESULTS AND DISCUSSION

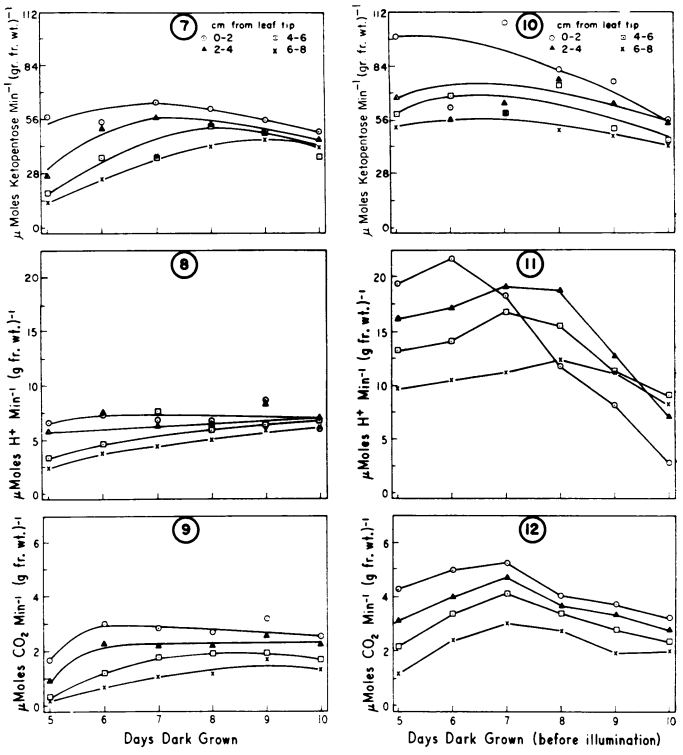
In terms of fresh weight or dry weight no significant growth of first leaf blades occurred in 48 hr after exposure of 7-day-old dark-grown seedlings to illumination at 10,760 lux. Because dark-grown leaves are rolled (18), leaf areas, measured by an air flow planimeter, could not be determined accurately by unfolding the leaves manually. After 12 hr of illumination the leaves were unfolded, and area was determined much more reliably (Fig. 1). Fresh weight per section, dry weight per section, and leaf area per section were maximal 4 to 12, 2 to 6, and 2 to 10 cm from the leaf tip, respectively.

The activity of each enzyme decreased from tip to base (Figs. 2, 3, 4). The increase in activity of phosphoriboisomerase due to illumination was nearly equal in all sections (Fig. 2). The increase in phosphoribulokinase activity due to illumination decreased steadily from the tip to the base (Fig. 3), whereas that of ribulose-1,5-diP carboxylase was constant for the tip 6 cm, below which it also steadily decreased from tip to base (Fig. 4). The carboxylase activity of the most basal section did not increase significantly with less than 48 hr of illumination.

Concentration of soluble protein decreased from tip to base,



FIGS. 1 TO 6. Distribution of area of 50 leaf sections, phosphoriboisomerase activity, phosphoribulokinase activity, ribulose-1,5-diP carboxylase activity, soluble protein, and total chlorophyll in different leaf sections after various periods of illumination. All seedlings were grown in darkness for 7 days before illumination at 10,760 lux. Standard errors of the difference between means of illumination periods within a section, and between section means within an illumination period, respectively, were 1.9, 2.6 (Fig. 1); 13.4, 12.0 (Fig. 2); 1.3, 1.2 (Fig. 3); 0.52, 0.41 (Fig. 4); 2.3, 2.0 (Fig. 5); and 0.11, 0.08 (Fig. 6).



FIGS. 7 TO 12. Effect of age of dark-grown seedlings on phosphoriboisomerase activity (Figs. 7, 10), phosphoribulokinase activity (Figs. 8, 11), and ribulose-1,5-diP carboxylase activity (Figs. 7, 12) in various leaf sections without illumination (Figs. 7, 8, 9) and after 24 hr of illumination (Figs. 10, 11, 12) at 10,760 lux, respectively. Standard deviations of the difference between day means within a section and between section means within a day, respectively, were 25.8, 18.2 (Fig. 7); 0.88, 0.84 (Fig. 8); 0.36, 0.29 (Fig. 9); 35, 31 (Fig. 10); 2.6, 1.5 (Fig. 11); and 0.44, 0.40 (Fig. 12).

and all sections increased similarly during illumination (Fig. 5). Accumulation of chlorophyll per g fresh weight was maximal 2 to 10 cm from the leaf tip, the section which coincided with maximal leaf area (Fig. 6).

These phenomena may be attributed to the manner in which barley leaves develop and the availability of substrates during development. Barley leaves develop from an intercalary meristem at the base of the blade (4). Leaf tips are differentiated first, followed by the development of sections toward the base. The expansion and development of leaf tips occur during a phase when endospermic carbohydrate is available as substrate. When the basal sections are fully elongated, dark-grown seedlings are beginning the phase of exhaustion of endospermic carbohydrate (8). Availability of an energy source for a longer time could allow greater physiological development in leaf tips than in basal sections. Rhodes and Yemm (13) reported that older cells near the tip of a 4-day-old leaf contained many plastids which developed rapidly during 24 hr of illumination whereas young cells at the base of the leaf contained smaller plastids which were less fully developed after the same illumination. During illumination, phosphoribulokinase activity (Fig. 3), ribulose-1,5-diP carboxylase activity (Fig. 4), and chlorophyll concentration (Fig. 6) increased most in the older cells of the leaf tip, whereas phosphoriboisomerase activity (Fig. 2) and soluble protein (Fig. 5) increased similarly in all sections. Thus, responses to illumination were greater in enzymes in the chloroplasts than in enzymes and other components not limited to the chloroplasts.

To determine the effect of age without illumination on the several parameters, leaf sections were harvested after 5 to 10 days of seedling growth in darkness. No significant growth of first leaf blades occurred in darkness after 7 days as measured by fresh weight. The tip 4 cm did not grow significantly after 5 days. Phosphoriboisomerase activity (Fig. 7) did not change significantly with increasing age in the tip 2 cm but did increase through 7 days in the tip 4 cm and then changed little thereafter. Activities in the 4- to 6- and 6- to 8-cm sections, respectively, increased through 8 and 9 days. Phosphoribulokinase activity (Fig. 8) changed little with age in the tip 4 cm but gradually increased in the 4- to 8-cm region during 10 days in darkness. Ribulose-1,5-diP carboxylase activity (Fig. 9) increased in the tip 4 cm until 6 days and in the 4- to 8-cm region until 7 days, and then remained constant.

To determine the effect of age on response to light, seedlings were grown in darkness for 5 to 10 days and then exposed to 24 hr of illumination at 10,760 lux (Figs. 10, 11, 12). By 8 days a slight loss of fresh weight and indication of wilting occurred in the 0- to 2-cm section but not in the other sections. These symtoms in the tip section became slightly more pronounced after illumination. Small necotic areas developed in the tip section (0-2 cm) following 24 hr of illumination of 9- and 10-day-old seedlings.

Phosphoriboisomerase activity after illumination changed little with increasing age except for a decrease in the tip 2 cm of the leaf (Fig. 10). The effect of age on the response of phosphoribulokinase activity to illumination differed in various sections of the leaf (Fig. 11). Responses were maximal in the first 2 cm of the leaf tip at 6 days, at 7 days in sections between 2 and 6 cm from the tip, and at 8 days in the 6- to 8-cm sections. Thereafter, activity in tip sections declined rapidly with age, shifting maximal activity toward the middle of the leaf with increased age prior to greening. Response of ribulose-1,5-diP carboxylase activity to illumination was maximal at 7 days (Fig. 12). The response with age was similar in all leaf sections, but activity was highest in tip

sections and progressively less in sections toward the base. Light-induced increases in soluble protein were greatest in 5- to 7-day-old seedlings (Fig. 13). After 7 days, soluble protein content declined more rapidly in leaf tips than in median sections. In dark-grown leaves without illumination, soluble protein content (Fig. 14) decreased with age through 7 days in the tip 2 cm of the leaf but increased in sections 2 to 8 cm from the tip. The highest enzymatic activities were generally associated with higher levels of soluble protein (compare Figs. 2–4 and 8–12 with Figs. 5, 13, and 14).

The capacity of barley leaves to accumulate chlorophyll upon illumination decreased with age. The capacity in leaf tips was maximum at 5 days and rapidly decreased with increasing age before illumination (Fig. 15). Chlorophyll accumulation after 7 days was maximum in the median sections (Fig. 6). The shift in distribution of chlorophyll accumulation was similar to that reported for protochlorophyll levels in etiolated barley leaves (17). At 8 days the levels of protochlorophyll declined from tip to base, but after 10 days the content at the tip had decreased, shifting maximal concentration to 1 to 3 cm from the tip.

Accumulation of chlorophyll depends upon available food reserves. Detached bean leaves, which had lost their ability to green as a result of the depletion of substrates, regained the capacity to synthesize chlorophyll after incubation overnight with 0.25 M sucrose (14). The depletion of seedling reserves in barley leaves grown in prolonged darkness may explain the decrease with aging in capacity for chlorophyll accumulation. Evidence of progressive senescence from tip to base after prolonged growth in darkness is the loss of greening capacity with increasing age (Fig. 15), loss of fresh weight, loss of turgor, and

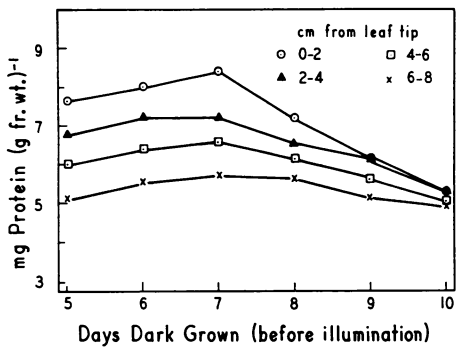


FIG. 13. Effect of age of dark-grown seedlings on the soluble protein content of cell-free extracts of various leaf sections after 24-hr illumination at 10,760 lux. Standard error of the difference between day means within a section was 3.4, and between section means within a day was 2.8.

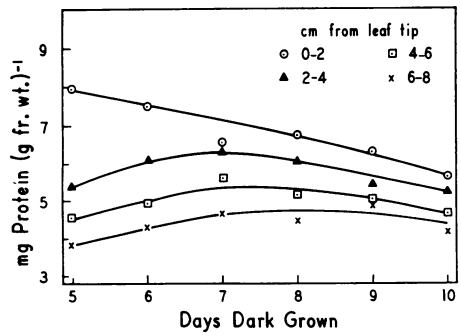


FIG. 14. Effect of age of dark-grown seedlings on soluble protein of cell-free extracts of various leaf sections without illumination. Standard error of the difference between day means within a section was 3.3 and between section means within a day was 3.1.

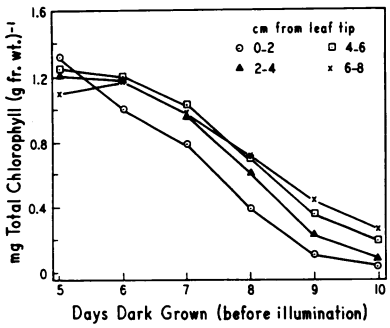


FIG. 15. Effect of age of dark-grown seedlings on accumulation of chlorophyll of various leaf sections after 24 hr of illumination at 10,760 lux. Standard error of the difference between day means within a section was 0.05, and between section means within a day was 0.05.

beginning of necrosis in leaf tips after 24 hr of illumination of 9- and 10-day-old seedlings.

The activities of phosphoriboisomerase (Fig. 2), phosphoribulokinase (Fig. 3), and ribulose-1,5-diP carboxylase (Fig. 4) correlated linearly with total chlorophyll (Fig. 6) for each light-duration treatment (r = 0.93, 0.99, and 0.99, respectively) and for each leaf section (r = 0.82, 0.84, and 0.74, respectively). Although light-initiated increases in enzymatic activities did not occur without chlorophyll accumulation, the relationship may still be indirect between activities of the carboxylative phase enzymes and accumulation of chlorophyll. Huffaker *et al.* (7) showed that the linear correlation of phosphoribulokinase activity with chlorophyll content was lost at low intensities of illumination. Benedict and Kohel (2) recently reported that the increase in activity of ribulose-1,5-diP carboxylase is not correlated with chlorophyll accumulation in virescent cotton leaves. Increases in activities of phosphoriboisomerase, phosphoribulokinase, and ribulose-1,5-diP carboxylase were not linearly correlated with chlorophyll synthesis at various temperatures in leaves of a temperature-sensitive chlorophyll mutant of alfalfa (6).

That the responses of phosphoriboisomerase, phosphoribulokinase, and ribulose-1,5-diP carboxylase to light, leaf age, and section are different is not surprising; other evidence suggests that a differential synthesis of these enzymes can occur. Levine and Togasaki (9) reported an ultraviolet-induced mutant strain of *Chlamydomonas reinhardi* which lacked ribulose-1,5-diP carboxylase but contained phosphoriboisomerase and phosphoribulokinase activities similar to those of the wild-type strain. The production of these enzymes also showed differing temperature optima in leaves of a temperature-sensitive chlorophyll mutant of alfalfa (6). Present evidence shows that fraction I protein is probably crude ribulose-1,5-diP carboxylase (15, 16). Fraction I protein may account for up to 50% of the soluble protein of green leaves (3) and is located in the chloroplast (11). Phosphoribulokinase and phosphoriboisomerase are apparently associated with fraction II protein (16), a heterogeneous soluble protein component ($s_{20,w}$ 3–4) of green leaves.

When dark-grown barley leaves are fully expanded, a number of physiological processes are in a state of change. At 7 days, significant growth of leaf blades is complete (7); respiration reaches a peak (5, 8, 20); and nucleotides, protein nitrogen (13). and soluble protein (Fig. 13) are at maximum concentration. Between 6 and 7 days of age, activities of phosphoribulokinase and ribulose-1,5-diP carboxylase increased maximally in response to illumination (Figs. 10, 11, 12).

In relation to seedling development, the reserve carbohydrates may be a major influence on the development of photosynthetic capacity. Prior to full elongation of first-leaf blades in darkness, seedling reserves may be utilized as substrates for the rapid

development of photosynthetic capacity. As reserves are depleted with prolonged dark growth, the ability to develop a high photosynthetic capacity during illumination diminishes steadily. The survival of developing barley seedlings may be influenced by the level of reserves during early exposure to illumination.

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