Chlorophyll, Ribulose-1,5-diphosphate Carboxylase, and Hill Reaction Activity in Developing Leaves of Populus deltoides

DONALD I. DICKMANN
Institute of Forest Genetics, North Central Forest Experiment Station, United States Department of Agriculture Forest Service, Rhinelander, Wisconsin 54501

ABSTRACT

The synthesis of chlorophyll and ribulose diphosphate carboxylase as well as the development of Hill reaction activity were followed in expanding Populus deltoides leaves and related to photosynthetic patterns. Total chlorophyll, which was not correlated with photosynthetic rate in expanding leaves, decreased slightly with age in young leaves, due to a decrease in chlorophyll b, but then increased linearly. The ratio of chlorophyll a to b, which rose sharply in young leaves, was highly correlated with the onset of net photosynthesis. Hill reaction activity was very low in young leaves and did not increase significantly until leaves were about half expanded. Ribulose diphosphate carboxylase activity increased in a sigmoid fashion with leaf ontogenesis and closely paralleled development of the photosynthetic system. The study demonstrates the importance of chlorophyll a and Calvin cycle enzyme synthesis to photosynthetic development in expanding leaves.

A comprehensive investigation of the developing apex of Eastern cottonwood (Populus deltoides Bartr.) is currently underway at this laboratory. As a basis for experiments in this expanding zone, the first unfolding leaf to reach 2 cm in length at the apex of a plant has been designated the index leaf and assigned a LPA 4 of 0. The expanding leaf zone extends downward approximately seven leaves from the index leaf, a leaf of LPA 7 being fully expanded (9). In the present study, selected biochemical factors associated with photosynthesis were investigated in the expanding leaf zone of young cottonwood plants. Chlorophyll content, RuDP carboxylase activity, and the Hill reaction were determined in leaves in various ontogenetic stages and their correlation with rates of CO₂ uptake noted.

MATERIALS AND METHODS

Cottonwood plants were raised from seed in controlled environment rooms in quartz sand cultures (8). When plants reached the 8- to 10-leaf stage, the cotyledons and first four small, linear leaves above them were excised to maintain uniformity. Plants used in the present study had 19 to 22 leaves.

CO₂ Exchange Measurements. Rates of net photosynthesis of individual leaves in the expanding zone of three plants were determined by monitoring CO₂ concentrations in an open gas circuit with an infrared gas analyzer (5). For each leaf, net CO₂ flux in light of saturating intensity (5.2 × 10⁴ ergs/cm² sec) was recorded for 10 min after a constant rate of CO₂ exchange had been attained.

Chlorophyll and RuDP Carboxylase Determinations. Individual leaves in the expanding leaf zone (LPA 0-7) of four different plants were excised, immediately weighed, and their lengths measured. The midrib was then removed and one-half of each leaf placed in a Duall tissue grinder containing 3 ml of 80% (v/v) acetone. The leaves were homogenized for 1 to 2 min and the homogenate was centrifuged at 1000g for 5 min. The supernatant fraction was decanted, and the sediment was washed once by centrifugation with 80% acetone. The supernatants were combined, brought to volume, and chlorophyll a and b determined spectrophotometrically (1). Chlorophyll determinations were also made for whole leaves on three additional plants.

The other one-half of each leaf used for chlorophyll determinations was immediately placed in a Duall tissue grinder with 3 ml of ice-cold 40 mm tris-HCl, pH 7.8, containing 10 mm MgCl₂, 0.25 mm EDTA, and 5.0 mm GSH (3). Each leaf sample was homogenized for 1 to 2 min, and the homogenate was centrifuged at 3000g for 20 min. All preparative procedures were carried out at 0 to 4 C. RuDP carboxylase activity was determined by adding 0.1 ml of the supernatant fraction to 0.4 ml of a mixture of 10 μm tris-HCl, pH 7.8, 2.5 μm MgCl₂, 0.1 μm EDTA, 1.25 μm GSH, 1.15 μm RuDP, and 2.5 μm NaH¹⁴CO₃ (0.1 μc/μmole). The mixture was incubated at 25 C for 5 min, and the reaction was stopped by adding 0.2 ml 5 M acetic acid. For each leaf extract two replications plus a blank without RuDP were run. The amount of ¹⁴C fixed was determined by counting 0.2-ml aliquots of the acidified reaction mixture in a liquid scintillation spectrometer.

Hill Reaction Determinations. Individual leaves in the expanding zone from four plants were excised, weighed, and homogenized as above. The extracting medium consisted of 50 mm sodium phosphate (pH 7.2), 10 mm KCl, 50 mm sucrose, and 2.5 mm MgCl₂. The extracts were centrifuged at 250g for 2 min, and the sediment was discarded. The supernatant was then centrifuged for 10 min at 1000g, and the pellet was washed once by centrifugation with phosphate buffer. All procedures were carried out at 0 to 4 C. Two milliliters of acetone were added to 0.5 ml of the resuspended chloroplast fragments and shaken. After centrifugation for 5 min at 1000g, total chlorophyll concentration of the supernatant was determined (1). The reaction mixture for determination of Hill activity contained chloroplast fragments (10-15 μg chlorophyll) in 2 ml of phosphate buffer plus 0.1 μmole DPIP. Photoreduction
of DPIP by chloroplasts in saturating light after 1 min was measured spectrophotometrically at 590 nm.

Because of the small size of leaves LPA 2 or younger (Table I), it was thought that RuDP carboxylase and Hill reaction activity might be too low to measure when individual leaves were assayed. Therefore, leaves in each of these developmental stages from four additional plants were combined in two separate runs in order to maximize enzyme and Hill activity. However, data from these determinations did not differ significantly from those in which only single leaves were assayed when expressed on a unit chlorophyll or fresh weight basis.

RESULTS AND DISCUSSION

The average length of leaves of the cottonwood plants used in this study increased from 2.3 cm at approximately LPA 0 to 10 cm at full expansion (LPA 7) (Table I). Leaf fresh weight averaged 41 mg at LPA 0 but increased rapidly to 1.178 mg at LPA 7.

Cottonwood leaves of LPA 0 and 1 exhibited little or no capacity for CO₂ fixation and evolved CO₂ in the light, whereas net photosynthetic uptake of CO₂ was observed by leaves LPA 2 or older (Fig. 1). Rates of net uptake increased rapidly between LPA 2 and 4 and then more slowly up to a maximum at LPA 7. The genesis of photosynthesis in expanding leaves of cottonwood plants of various ages has been discussed in detail by Dickmann (5).

The relationship of chlorophyll content to photosynthesis as leaves mature and age has long been the subject of investiga-

Table 1. Mean Length and Fresh Weight of Developing Cottonwood Leaves Used for Determination of Chlorophyll, RuDP Carboxylase, and Hill Reaction Activity

<table>
<thead>
<tr>
<th>Approximate LPA</th>
<th>Leaf Length</th>
<th>Leaf Fresh Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.3</td>
<td>41</td>
</tr>
<tr>
<td>1</td>
<td>3.1</td>
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<td>8.9</td>
<td>862</td>
</tr>
<tr>
<td>6</td>
<td>9.6</td>
<td>1047</td>
</tr>
<tr>
<td>7</td>
<td>10.0</td>
<td>1178</td>
</tr>
</tbody>
</table>

Fig. 1. Net CO₂ exchange by developing cottonwood leaves in saturating light. CO₂ concentrations in an open gas circuit were monitored for 10 min with an infrared gas analyzer.

FIG. 2. Total chl a, chl b, and chl a/b ratios of 80% acetone extracts from developing leaves of cottonwood.

FIG. 3. Hill reaction activity in isolated chloroplasts from developing leaves of cottonwood. Chloroplast photoreduction of DPIP after 1 min was determined spectrophotometrically at 590 nm.

Fig. 4. RuDP carboxylase activity in developing leaves of cottonwood. Soluble protein extracts were incubated in the presence of RuDP and NaH¹⁴CO₃ for 5 min, and the amount of ¹⁴CO₂ fixed was determined by liquid scintillation spectrometry.
tion by plant physiologists (7, 10, 12, 13, 16). Although some of these studies have shown a direct relationship between chlorophyll content and photosynthesis, very young, nonphotosynthesizing leaves have not been included. In the present study cottonwood leaves of LPA 0 had little, if any, capacity for fixation of CO₂ yet they possessed considerable total chlorophyll (Fig. 2). As leaves matured, the proportional rise in the rate of photosynthesis was significantly greater than the rise in total chlorophyll. Furthermore, when photosynthetic rates stabilized in mature leaves (LPA 6 and 7), chlorophyll concentration continued to increase at a rapid rate (Fig. 2). Thus, a close relationship between photosynthesis and total chlorophyll content is lacking in expanding cottonwood leaves.

When chlorophyll a to b ratios are compared with the ontogenic increase in photosynthetic rate a closer relationship is noted, however. The ratio of chlorophyll a to b increased strikingly during early stages of leaf development, first reaching a maximum at LPA 2 (Fig. 2), the same stage that net photosynthesis was first observed. The increase in a/b ratio, a phenomenon also noted by Sestak (11) in spinach and radish leaves, was due primarily to an increase in the chlorophyll a component in the present study, probably reflecting synthesis of an active form of this pigment.

Hill reaction activity in chloroplasts isolated from cottonwood leaves of LPA 0 to 3 was very low but increased rapidly in leaves LPA 4 or older (Fig. 3). Thus, the rapid rise in chlorophyll a/b ratio preceded by several plastochrons the development of Hill activity in the critical zone where photosynthetic CO₂ uptake increased rapidly. A similar lag in Hill activity was noted by Thorne and Boardman (17) in green pea leaves. They concluded that during chloroplast development there is present in the plastid some chlorophyll which has not been incorporated into the pigment assemblies of either photosystem I or II. The genesis of the energy conversion system of maturing cottonwood leaves in the present study might then be envisaged as a two step sequence: rapid initial synthesis of chlorophyll a followed by assembly of the grana membranes in the chloroplasts, with the concomitant increase in Hill activity.

RuDP carboxylase, the major enzyme responsible for the initial fixation of CO₂ in photosynthesis, is localized in the chloroplasts of higher plants (6, 15) and it may constitute 5 to 10% of the total soluble protein of leaves (19). Thus, this enzyme should be highly correlated with net photosynthesis in developing leaves, a statement borne out by the present study (Fig. 4). RuDP carboxylase levels were low in young cottonwood leaves incapable of net photosynthesis, whereas the development of enzyme activity closely paralleled the increase in photosynthetic rate in older leaves (Fig. 4). These findings are in agreement with those of Smillie (14) for growing pea leaves and Bradbeer (4) for greening bean leaves.

In contrast, a close relationship of RuDP carboxylase to total chlorophyll in expanding leaves has not been established (2). Similar results were shown by the present study, particularly in later stages of leaf development when total chlorophyll increased at a faster rate than did RuDP carboxylase. Furthermore, the development of Hill activity in chloroplasts isolated from cottonwood leaves lagged behind RuDP carboxylase activity in the zone where net photosynthesis increased rapidly. Togasaki and Levine (18) found that a strain of the green alga Chlamydomonas reinhardtii, which possessed mutations that blocked photosynthetic electron transport, nevertheless had normal RuDP carboxylase levels. These results and data of the present study indicate that the absence of an intact photosynthetic electron transport chain has no effect on the level of RuDP carboxylase.

The present study demonstrates the importance of protein metabolism to the onset of photosynthesis during leaf ontogenesis in cottonwood. Apparently, the photosynthetic mechanism of newly formed leaves is inoperative until critical levels of Calvin cycle enzymes are synthesized, even though considerable total chlorophyll may be present. The increase in enzyme activity, paralleled by a rapid synthesis of chlorophyll a, is followed, after several plastochrons, by a rise in Hill activity as pigment molecules are incorporated into the chloroplast membranes.

Acknowledgments—The author wishes to express his thanks to Drs. Philip Larson and John Gordon for their advice and suggestions during the course of these experiments.

LITERATURE CITED