Activation of NADP-Malate Dehydrogenase, Pyruvate, Pi Dikinase, and Fructose 1,6-Bisphosphatase in Relation to Photosynthetic Rate in Maize

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ABSTRACT

The activity and extent of light activation of three photosynthetic enzymes, pyruvate, Pi dikinase, NADP-malate dehydrogenase (NADP-MDH), and fructose 1,6-bisphosphatase (FBPase), were examined in maize (Zea mays var Royal Crest) leaves relative to the rate of photosynthesis during induction and under varying light intensities. There was a strong light activation of NADP-MDH and pyruvate, Pi dikinase, and light also activated FBPase 2- to 4-fold. During the induction period for whole leaf photosynthesis at 30°C under high light, the time required to reach half-maximum activation for all three enzymes was only 1 minute or less. After 2.5 minutes of illumination the enzymes were fully activated, while the photosynthetic rate was only at half-maximum activity, indicating that factors other than enzyme activation limit photosynthesis during the induction period in C4 plants.

Under steady state conditions, the light intensity required to reach half-maximum activation of the three enzymes was similar (300-400 microEinsteins per square meter per second), while the light intensity required for half-maximum rates of photosynthesis was about 550 microEinsteins per square meter per second. The light activated levels of NADP-MDH and FBPase were well in excess of the in vivo activities which would be required during photosynthesis, while maximum activities of pyruvate, Pi dikinase were generally just sufficient to accommodate photosynthesis, suggesting the latter may be a rate limiting enzyme.

There was a large (5-fold) light activation of FBPase in isolated bundle sheath strands of maize, whereas there was little light activation of the enzyme in isolated mesophyll protoplasts. In mesophyll protoplasts the enzyme was largely located in the cytoplasm, although there was a low amount of light-activated enzyme in the mesophyll chloroplasts. The results suggest the chloroplastic FBPase in maize is primarily located in the bundle sheath cells.

In C4 plants, light intensity is normally considered a major limiting factor for photosynthesis under optimum temperature. Light drives several processes in C4 photosynthesis, including the C4 cycle, provision of energy to the RPP pathway, and the light activation of several enzymes. Under limiting light, it is not clear if all of these factors are co-limiting or if there is one primary limitation.

NADP-MDH and pyruvate, Pi dikinase of the C4 cycle are light activated in C4 plants, and PEP carboxylase is also activated by light to some extent (8, 11, 21). Several enzymes of the RPP pathway are light activated in C4 plants (fructose 1,6-bisphosphatase, sedoheptulose 1,7-bisphosphatase, phosphoribulokinase and NADP-triose-P dehydrogenase) (1, 3) but there is little evidence that these enzymes are strongly modulated by light in vivo in C4 plants. Steiger et al. (23) generally found little light activation of NADP-triose-P dehydrogenase and phosphoribulokinase in C4 plants compared to C3 plants. Björkman and Badger (2) found a several-fold activation of NADP-triose-P dehydrogenase and phosphoribulokinase in the C4 species Tydestronia oblongifolia but no light activation of FBPase. In addition, activation of photosynthetic enzymes relative to rates of leaf photosynthesis in C4 plants has not been evaluated. In the present study, the activity and extent of activation of NADP-MDH, pyruvate, Pi dikinase, and FBPase in the C4 plant maize was examined relative to photosynthetic rate during induction and under varying light intensities.

MATERIALS AND METHODS

Material. Seeds of Zea mays (hybrid sweet corn, var Royal Crest) were obtained from Olds Seed Co., Madison, WI. Plants were grown in controlled chambers with a 14-h light/10-h dark period, with day/night temperatures of 27°C/22°C. The light intensity was 700 μE m⁻² s⁻¹, except in Figure 2 (see legend) in which the light intensity during growth was 400 μE m⁻² s⁻¹ for some experiments. Plants were grown in a commercial premix (55% peat moss, 30% pumice, and 15% sand) and watered with a dilute nutrient solution three times a week according to Schmitt and Edwards (20). The largest expanded leaves of plants approximately 3 weeks of age were used in the experiments.

Gas Exchange Measurements. CO₂ exchange of intact individual leaves was measured according to Monson et al. (17), with an open IR gas analysis system under 330 μl/CO₂ in air. Plants were incubated in a growth chamber at 30°C. A single leaf was placed in a cuvette and maintained at 30 + 0.5°C using a Peltier-cooled heat exchanger. Varying levels of irradiance were achieved by changing the number of cheesecloth screens between the leaf chamber and light source. The light source was a 1-kw metal halide lamp which was used both for the gas exchange measurements and enzyme analyses (see below). Light intensities were measured with a quantum sensor (model 1776 from Li Cor Instruments) mounted within the leaf chamber adjacent to the enclosed leaf.

Preparation of Leaf Tissue for Enzyme Assays. Plants were
incubated in a growth chamber at 30°C. At the same time gas exchange measurements were made on a single leaf, samples were taken from other leaves for enzyme assays (see legends for details of sampling). A low intensity, green safe light was used for samples taken in the dark (18). The leaf samples were frozen and stored in liquid nitrogen until extraction for enzyme assay. For FBPass and NADP-MDH assays, the frozen leaf samples were rapidly homogenized using a chilled mortar with 5 mg insoluble PVP and 0.2 g of acid-washed sand plus 0.5 ml of ice-cold medium containing 100 mM Tris-HCl (pH 7.8), 1 mM MgCl₂, 0.1 mM EDTA, 5 mM DTE, 0.5% (v/v) Triton X-100, and 0.5% (w/v) BSA. Without a reducing agent (e.g., DTE), FBPass (J. Kobza and G. E. Edwards, unpublished data) and NADP-MDH (18) rapidly lose their original in vivo state of activation. By extracting at low temperature and assaying within minutes, no further activation occurs in vitro. For pyruvate, Pi dikinase, 5 mg insoluble PVP and 0.2 g of sand, and 0.5 ml of ice-cold medium containing 50 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 1 mM EDTA, 2.5 mM pyruvate, 10 mM DTE, and 0.5% (w/v) BSA were used for homogenization. After homogenization, 1.5 ml of the same medium was added. The homogenates were filtered through Miracloth. An aliquot of the filtrate was taken for Chl determination. For the assay of FBPass and pyruvate, Pi dikinase, the homogenate was centrifuged at 14,000g for 1 min and the supernatant was used as the crude enzyme extract. For the assay of NADP-MDH, a 25 μl aliquot of the homogenate was added to 100 mM Tris-HCl (pH 8.0), 0.5 mM EDTA, and 0.2 mM NADP (total volume of 1 ml), and centrifuged at 14,000g for 1 min. The supernatant was immediately used for enzyme assay.

Isolation of Mesophyll Protoplasts and Bundle Sheath Strands. Mesophyll protoplasts and bundle sheath strands were enzymically isolated according to previous methods using a combination of cellulase and Macerozyme (6, 10). The preparations were suspended in a medium containing 50 mM Hepes-KOH (pH 8.0), 0.4 M sorbitol, and 5 mM EDTA.

Isolation of Mesophyll Chloroplasts. Mesophyll protoplasts were suspended in a medium containing 0.4 M sorbitol, 50 mM Hepes-KOH (pH 7.6), and 1 mM EDTA, and ruptured by three passes through a 20-μm nylon net attached to a 1-ml syringe, as previously described (6). The protoplast extracts were centrifuged for 1 min at 650g to pellet the chloroplasts. The pellet was resuspended in the medium containing 0.4 M sorbitol, 50 mM Hepes-KOH (pH 8.0), and 5 mM EDTA.

Extraction of Mesophyll and Bundle Sheath Preparations for Enzyme Assays. After light or dark treatment, mesophyll protoplasts, mesophyll chloroplasts, and bundle sheath strands, suspended in plastic tubes in a medium containing 0.4 M sorbitol, 50 mM Hepes-KOH (pH 8.0), 5 mM EDTA, and 10 mM NaHCO₃, were transferred into liquid nitrogen for storage prior to assay. The frozen preparations were thawed with addition of an equal volume of medium containing 200 mM Tris-HCl (pH 7.8), 7 mM MgCl₂, 0.2 mM EDTA, 10 mM DTE, 0.2% (w/v) Triton X-100, and 1% (w/v) BSA. In the case of mesophyll protoplasts and mesophyll chloroplasts, an aliquot was taken from each suspension for Chl determination, the remainder was centrifuged at 14,000g for 10 s and the supernatant was used for enzyme assays. Bundle sheath strands were treated in a similar manner, except they were broken with an ice-cold Teflon-glass homogenizer after addition of the medium. An aliquot of the homogenized suspensions were saved for the Chl determination and the rest were centrifuged at 14,000g for 10 s and the supernatant used for assay of enzymes. The FBPass assay was initiated within 3 min after thawing of the preparations.

Assay of Enzyme Activities and Chl Determination. NADP-MDH was assayed as previously described (18) at 30°C in a medium containing 100 mM Tris-HCl (pH 8.0), 0.5 mM EDTA, 0.2 mM NADPH, and 2 mM oxaloacetate. The assay was initiated by addition of oxaloacetate.

The assay mixture for FBPass contained 100 mM Tris-HCl (pH 7.8), 5 mM MgCl₂, 0.4 mM NADP, 0.5 mM EDTA, 4 units of glucose-P isomerase, and 2 units of glucose-6-P dehydrogenase. The reaction was initiated by addition of FBP to a final concentration of 0.25 mM. A relatively low concentration of FBP was used since higher concentrations of FBP gave less difference between the activity from darkened versus illuminated leaves (also in C₅ plants the light-activated form of FBPase has a lower Km for the substrate than the dark form). The activity of nonspecific phosphatase, which was low or undetected in the preparations, was estimated by omission of MgCl₂.

Pyruvate, Pi dikinase and NADP-triose-P dehydrogenase were measured as previously described by Nakamoto and Edwards (18) and Spalding et al. (22), respectively.

The assay medium for PEP carboxylase contained 50 mM Hepes-KOH (pH 8.0), 5 mM NaHCO₃, 0.2 mM NADH, 10 mM MgCl₂, and 5 units of malate dehydrogenase. The reaction was initiated by adding PEP to a final concentration of 2.5 mM.

The assay mixture for NADP-malic enzyme contained 50 mM Hepes-KOH (pH 8.0), 5 mM EDTA, 5 mM DTE, 0.5 mM NADP, and 5 mM K-malate (pH 7.0). The reaction was initiated by adding MgCl₂ to a final concentration of 22.5 mM.

Chl was determined according to Wintermans and De Mots (26).

RESULTS

In maize leaves, FBPass was activated in the light and inactivated in the dark, with the degree of light activation 2- to 4-fold the activity in darkened leaves (Fig. 1B; Table I). The in vivo activity of NADP-MDH and pyruvate, Pi dikinase in maize is strongly dependent on light (Fig. 1, A and C; Hatch [8]). The time course of activation of NADP-MDH and pyruvate, Pi dikinase in the light was similar to that of FBPass (Fig. 1, A-C). Also, the rate of dark inactivation of NADP-MDH and FBPass was similar. Dark inactivation of pyruvate, Pi dikinase was not measured (the effect of varying environmental conditions on the dark inactivation of pyruvate, Pi dikinase and NADP-MDH has been recently studied (18)). The half-time for the light activation of the three enzymes was about 0.5 to 1 min. The time required to reach half-maximum rate of leaf photosynthesis was about 2.5 min, at which time the three enzymes had reached maximum level of activation.

The influence of light intensity on the degree of activation of NADP-MDH, pyruvate, Pi dikinase, and FBPass, and on the rate of leaf photosynthesis in maize, is shown in Figure 2. At each light intensity, leaves were preilluminated for 20 min to assure steady state conditions had been reached (preliminary experiments at both high and low light intensities indicated steady state conditions were obtained within 2 to 3 min for the light activation of enzymes and within 20 min for photosynthetic rates). A light intensity of 300 to 400 μE m⁻² s⁻¹ was required for half-maximum activation of all three enzymes. Approximately 550 μE m⁻² s⁻¹ of light was required to give half-maximum saturation of the photosynthetic rate and relatively high light intensity was required for photosynthesis to approach a maximum level. The light response curve for photosynthesis was similar for plants grown at the two light intensities (400 versus 700 μE m⁻² s⁻¹) and there was no apparent difference in the light required for activation of the pyruvate, Pi dikinase (plants grown at 400 μE m⁻² s⁻¹) and NADP-MDH and FBPass (plants grown at 700 μE m⁻² s⁻¹) (Fig. 2). In addition, outdoor grown plants had a similar light response curve for photosynthesis (half maximum activity at 600 μE m⁻² s⁻¹) and activation of pyruvate, Pi dikinase (half-maximum activation at 300 μE m⁻² s⁻¹) and NADP-MDH (half-maximum activation at 350 μE m⁻² s⁻¹), although rates of
photosynthesis and the pyruvate,Pi dikinase activity were slightly higher than in the growth chamber plants (data not shown).

It is known from previous work that pyruvate,Pi dikinase and NADP-MDH are located in the mesophyll chloroplasts of C₄ plants (5, 8). To determine the intercellular location of the light-activated FBPase, mesophyll protoplasts and bundle sheath strands were isolated from maize and preincubated in the dark or light for 20 min at 30°C prior to enzyme extraction and assay. Both mesophyll and bundle sheath preparations treated in the dark had FBPase activity (Table I). Light-treated bundle sheath strands showed a large increase in FBPase activity (about 5-fold), while light-treated mesophyll protoplasts showed only a slight increase in activity of the enzyme. DCMU, an inhibitor of noncyclic electron flow, at 10 μM levels prevented the light-dependent increase in activity in the preparations. Assay of PEP carboxylase (marker for mesophyll protoplasts) and NADP-malic enzyme (marker for bundle sheath cells) showed that good separation of the cell types was obtained.

Mesophyll protoplasts were fractionated and the chloroplast and extrachloroplastic fractions separated to determine the intracellular location of FBPase (Table II). Based on the distribution of the chloroplastic NADP-triose-P dehydrogenase among two preparations, the chloroplasts were 93 to 95% intact. The distribution of the cytosolic enzyme PEP carboxylase between the chloroplast pellet and supernatant indicated that only about 4% of the cytosolic fraction contaminated the chloroplast pellet. In these dark-treated preparations, the FBPase was totally located in the extrachloroplastic fraction (the low percentage of activity in the chloroplast pellet of 1 to 3% can be accounted for by cytosolic contamination). The activity of NADP-malic enzyme in the mesophyll preparations (Table II) was only 1% of that in bundle sheath cells (see Table I). Thus, the contribution of FBPase activity from bundle sheath contamination is insignificant (1% of the activity of FBPase from dark-treated cells, Table I, is only 0.3 μmol mg⁻¹ Chl h⁻¹). The low activity of NADP-malic enzyme in the mesophyll preparation was extrachloroplastic, which suggests any contaminating bundle sheath chloroplasts were broken since NADP-malic enzyme is located in the bundle sheath chloroplasts in C₄ plants (5).

When mesophyll chloroplasts isolated from protoplasts were illuminated, the activity of FBPase increased to 8 to 11 μmol mg⁻¹ Chl h⁻¹, compared to activity of less than 1 μmol mg⁻¹ Chl h⁻¹ in the dark-treated chloroplasts. DCMU strongly inhibited the light-dependent activation. Also, PGA, which uses ATP and reductive power during its reduction to glyceraldehyde-3-P, inhibited the light activation of FBPase in the mesophyll chloroplasts (Table III).

**DISCUSSION**

**Induction of Photosynthesis and Light Activation of Enzymes in Maize, a C₄ Plant.** Although light is known to be required for activation of NADP-MDH and pyruvate,Pi dikinase of the C₄ cycle in C₄ plants, measurements on enzyme activation and photosynthesis under varying conditions have not been studied. In the present study, when maize leaves were illuminated at 30°C, within 2.5 min the enzymes NADP-MDH, pyruvate,Pi dikinase, and FBPase had reached a maximum degree of activation, although photosynthesis had reached only half its maximum rate (Fig. 1). This finding indicates that, during induction, factors in addition to enzyme activation are critical for photosynthesis in C₄ plants to reach maximum capacity. Following illumination, photosynthesis may be limited until certain metabolites of the C₄ cycle and RPP pathway build up to a steady state level. Leegood (13) found that substantial changes occur in the levels of several metabolites of carbon assimilation over several minutes following a dark/light transition with maize. In particular, there was a large buildup of pyruvate, 3-P glycerate and dihydroxyacetone-P over the first 10 to 40 min of illumination. In C₄ plants enzyme activation may occur well before buildup of metabolites and increases in concentrations of metabolites of the C₄ cycle and RPP pathway may be important during induction. In addition, it takes about 15 min for full opening of stomata of maize leaves when cut leaves are illuminated (1500 μE m⁻² s⁻¹) following a 30-min period of darkness. However, calculations of the intercellular [CO₂] during induction indicate CO₂ is not limiting to photosynthesis (H. Usuda, unpublished data).

During induction of photosynthesis in C₄ plants, it has long been considered that light activation of enzymes and/or buildup of metabolites may be important factors (7). A current view on induction of photosynthesis in C₄ species links enzyme activation, specifically activation of FBPase, and changes in metabolite levels. Based on studies with isolated chloroplasts and the enzyme FBPase, it is suggested that an increase in the ratio of organic-P/Pi in the chloroplast may be required for the enzyme to reach maximum level of activation (9, 12, 15). Maximum degree of activation of FBPase appears to require both reductive conditions
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Table 1. Activity of Fructose 1,6-Bisphosphatase in Whole Leaf, Mesophyll Protoplasts, and Bundle Sheath Strands of Maize

<table>
<thead>
<tr>
<th>Material</th>
<th>Light</th>
<th>Fructose 1,6-bisphosphatase</th>
<th>Dark</th>
<th>PEP Carboxylase</th>
<th>NADP-malic Enzyme</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Light + 10 μM DCMU</td>
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<tr>
<td>Leaf</td>
<td>292</td>
<td>63.5</td>
<td>102</td>
<td>947</td>
<td>11.3</td>
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<tr>
<td></td>
<td>201</td>
<td>51.1</td>
<td>44.1</td>
<td>1140</td>
<td>19.5</td>
</tr>
<tr>
<td>Mesophyll protoplasts</td>
<td>58.6</td>
<td>54.4</td>
<td>41.5</td>
<td>1140</td>
<td>19.5</td>
</tr>
<tr>
<td></td>
<td>55.9</td>
<td>54.4</td>
<td>41.5</td>
<td>1140</td>
<td>19.5</td>
</tr>
<tr>
<td>Bundle sheath strands</td>
<td>156</td>
<td>32.5</td>
<td>29.0</td>
<td>0</td>
<td>1172</td>
</tr>
<tr>
<td></td>
<td>203</td>
<td>42.2</td>
<td>31.8</td>
<td>7.3</td>
<td>1413</td>
</tr>
</tbody>
</table>

Three separate extracts were made on the whole leaf, while two separate replications were made on the mesophyll and bundle sheath preparations. Whole plants were illuminated (1500 μE m⁻² s⁻¹) for 20 min or kept in the dark at room temperature prior to extraction. Mesophyll and bundle sheath preparations were preilluminated (1500 μE m⁻² s⁻¹) or kept in the dark at 30°C for 20 min prior to extraction. DCMU was added as ethanol solution (0.9% final concentration). Ethanol at this concentration does not affect the rate of photosynthetic ¹⁴C0₂ fixation by isolated bundle sheath cells (25).

and the presence of the substrate fructose 1,6-bisP. Buildup of organic-P outside the chloroplast can be important during induction of photosynthesis with isolated chloroplasts of C₃ plants. However, from studies with protoplasts and leaves of the C₄ species spinach, no substantial differences are found in dark versus light in the total pool of organic-P (24), so it was concluded that buildup of the total pool of organic-P may not occur in vivo. Rather, in leaves of C₃ plants, activation of enzymes and changes in the relative concentrations of certain organic-P within the chloroplast (e.g. ribulose-1,5-bisP and fructose-1,6-bisP) may be important during induction. In both C₃ and C₄ plants there is still insufficient data with intact leaves on activation of various enzymes, changes in metabolite pools, and rates of photosynthesis to make complete conclusions about the induction process.

Influence of Light Intensity on Photosynthesis and Light Activation of Enzymes in Maize. The light-response curves for activation of NADP-MDH, pyruvate, Pi dikinase and FBPase were similar to the light-response curve for photosynthesis, although higher light intensity was required to reach maximum rates of photosynthesis. While light activation of enzymes in both the C₃ cycle and RPP pathway is required for C₄ photosynthesis, light activation of enzymes in C₃ plants may serve as more than an on/off switch for certain enzymes between dark/light. If serving only as an on/off switch, full activation of the enzymes might occur at very low light intensities, well below that required to reach maximum rates of photosynthesis. In C₃ plants, light activation of enzymes may regulate the flux of carbon through various phases of carbon assimilation, or the degree of light activation of enzymes may limit the rate of photosynthesis at a given light intensity. The activity of NADP-malate dehydrogenase is well in excess of photosynthetic rate, so the enzyme would not appear rate-limiting. The activity of FBPase due to light activation is equivalent to the rate of leaf photosynthesis (Figs. 1, 2; Table I). In vivo, the chloroplastic FBPase needs to function at one-third the rate of CO₂ fixation during the regeneration of RuBP. Additional activity of FBPase would be required in the chloroplast if the product of photosynthesis is converted to starch (maximum of one-sixth of the rate of CO₂ fixation). The chloroplastic FBPase would therefore need to function, at maximum, up to one-half the rate of CO₂ fixation. Since the activity of FBPase due to light activation was approximately equivalent to the rate of photosynthesis under a given condition (Figs. 1, 2; Table I), the chloroplastic FBPase would appear more than sufficient to accommodate CO₂ assimilation. However, pyruvate, Pi dikinase activity was similar to the photosynthetic rate at a given light intensity (Fig. 2; H. Usuda, M. S. B. Ku, and G. E. Edwards, unpublished data with plants grown at different light intensities). Therefore, pyruvate, Pi dikinase may be a rate limiting enzyme for C₄ photosynthesis in vivo. Other enzymes, like chloroplastic FBPase and NADP-MDH, can apparently function in vivo below substrate saturation and accommodate rates of carbon assimilation.

In leaves of wheat, a C₃ plant, FBPase reached one-half maximum degree of activation at about 18 w m⁻² (4% of full sunlight), and full saturation at 60 w m⁻² (about 12% of full sunlight) (14). As FBPase reached one-half maximum degree of activation at 300 μE m⁻² s⁻¹ (about 15% full sunlight) and full activation at 700 μE m⁻² s⁻¹ (about 35% of full sunlight) in maize (Fig. 2), it appears much higher light intensity may be required to activate the enzyme in C₃ plants than in C₄ plants.

Characteristics of FBPase in Maize. In the C₃ plant maize, the present study shows a several-fold activation of FBPase in the light and inactivation in the dark, which is similar to that reported in the C₃ plant wheat (14). This is in contrast to results of Björkman and Badger (2) in which no light activation of FBPase was found in leaves of the C₄ plant T. oblongifolia. Either conditions used during extraction may mask the light activation of the enzyme or there may be species differences. In C₃ plants, FBPase is located in the chloroplast and cytosol of mesophyll cells, with the FBPase of the chloroplast being light activated (1, 2). The activity of FBPase obtained from darkened leaves of maize in the present study is similar to that found in darkened leaves of C₃ plants (14; J. Kobza and G. E. Edwards, unpublished data) which may be predominantly that of the cytosolic enzyme. In darkened mesophyll protoplasts of maize the enzyme activity was only found in the extrachloroplastic fraction following disruption of the protoplasts.

Maize leaves have a thioredoxin-linked FBPase and the enzyme can be activated in vitro by a reducing agent (DTT or photochemically from spinach thylakoids) and thioredoxin f (19). The present study indicates FBPase is light activated in vivo in maize, apparently in both the mesophyll and bundle sheath chloroplast by a mechanism similar to that in C₃ plants. With the isolated mesophyll and bundle sheath preparations it was found that both cell types had substantial activity of FBPase in the dark, but the main part of the light-activated FBPase was in the bundle sheath cells. It appears that both cell types may have significant amounts of cytosolic FBPase and that the chloroplas-
tions in the maize leaf, it is reasonable that the chloroplastic form of FBPase would be primarily located in the bundle sheath cell. Low activity of a light-activatable FBPase was found in the mesophyll chloroplast of maize. Under continuous illumination starch is synthesized in mesophyll as well as bundle sheath chloroplasts of maize (4). Therefore, under some circumstances the low activity FBPase in the mesophyll chloroplasts may function in starch synthesis, utilizing organic-P synthesized in bundle sheath chloroplasts. Presumably, the cytosolic FBPase in maize would be involved in metabolism of triose-P to sucrose.

Reducing equivalents are required for activating the thior- doxin-linked FBPase in maize (19). Lack of complete inhibition of activation by PGA or DCMU in the mesophyll chloroplasts (Table III) may be related to the fact that the capacity for the Hill reaction in these chloroplasts is quite high (7) while the FBPase activity in these chloroplasts is relatively low. On the other hand, in maize bundle sheath chloroplasts the activity of the Hill reaction is quite low in comparison to maize mesophyll and C₃ plant mesophyll chloroplasts (7, 16, 25). However, it appears there is sufficient redundant generated from the Hill reaction in bundle sheath chloroplasts of maize to activate FBPase (Table II), and redundant from other sources may not be necessary (i.e. from malate decarboxylation through NADP-malic enzyme).

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LITERATURE CITED

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