Limiting Factors in Photosynthesis

III. EFFECTS OF IRON NUTRITION ON THE ACTIVITIES OF THREE REGULATORY ENZYMES OF PHOTOSYNTHETIC CARBON METABOLISM

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SCOTT E. TAYLOR, NORMAN TERRY, AND ROBERT P. HUSTON

Department of Plant and Soil Biology, University of California, Berkeley, California 94720

ABSTRACT

When Fe was withheld from sugar beets (Beta vulgaris L. cv F58-44H1), the activities of NADP-glyceraldehyde-3-phosphate dehydrogenase, fructose 1,6-bisphosphatase, and ribulose 5-phosphate kinase were not diminished, while chlorophyll per area was decreased by 75%. On resupplying Fe, chlorophyll per area increased to control levels within 5 days, whereas the activities of the three enzymes remained approximately constant. These results support the view advanced earlier (Terry 1980 Plant Physiol 65: 114-120) that the photosynthetic effects of Fe deprivation are mediated by changes in the lamellar components of chloroplasts and not by changes in stromal enzymes involved in photosynthetic carbon reduction.

In an earlier paper of this series, evidence was presented that Fe stress influenced photosynthesis mainly by altering the capacity of leaves for light harvesting and electron transport (photochemical capacity) (23). Subsequently, Spiller and Terry (19) showed that Fe stress reduced the chloroplastic content of Chl a, Chl b, P700, and Cyt f without changing the ratios of Chl/P700 or Chl/Cyt f and that these events were accompanied by a drastic reduction in the thylakoid system. Based on the fact that severe Fe stress reduced RuBP1 carboxylase extractable activity by 30% while reducing Chl (a + b), P700, and Cyt f and photosynthesis by more than 90%, it was suggested that Fe stress may have relatively little effect on the stromal enzymes involved in photosynthetic carbon reduction, and if so, that Fe stress could be utilized as an experimental technique to study photosynthetic limitation (23).

The objective of the present investigation was to test the hypothesis that the influence of Fe nutrition on the enzymes of photosynthetic carbon reduction was small relative to the effects on the lamellar components. The approach used was to determine the influence of Fe nutrition on the activity of three important enzymes of the Calvin cycle: GPDHase, FBPase, and RPKase. These enzymes represent possible regulatory steps in CO2 assimilation: GPDHase catalyzes the reductive step that requires light-generated production of NADPH; FBPase has been suggested to have a pivotal role in the regulation of RuBP carboxylase (6); and RPKase catalyzes the regeneration of the CO2 acceptor, RuBP, and may be important in the regulation of photosynthetic carbon reduction (8).

1 Abbreviations: RuBP, ribulose 1,5-bisphosphate; FBPase, fructose 1,6-bisphosphatase (EC 3.1.1.11); GPDHase, NADP-glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.13); RPKase, ribulose-5-phosphate kinase (EC 2.7.1.19).

MATERIALS AND METHODS

Plant Culture. Sugar beets (Beta vulgaris L. cv F58-44H1) were grown hydroponically in growth chambers as described previously (22). Iron-deficient plants were obtained by transferring to culture solutions without Fe for a period of 7 d. Iron resupply studies were carried out by transferring Fe deficient plants to half Hoagland solution containing ferric-sodium EDTA. All determinations were done with rapidly expanding leaves as described previously (23).

Chloroplast Isolation. Leaves were harvested 2 to 3 h after the start of the photoperiod, and discs removed for determination of leaf Chl content. The remaining leaf material (after removing the mid-rib and petiole) was lowered in temperature by bathing in ice-cold distilled H2O for 5 min; three to four leaves (approximately 15 g of tissue) were used for each isolation.

Chloroplasts were isolated at 2°C by a 5 s homogenization in a Waring blender using the isolation medium: 20 mM Tricine (pH 7.6), 0.4 mM sorbitol, 10 mM NaCl, 10 mM MgCl2, 2 mM CaCl2, 2 mM MnCl2, 5 mM EDTA, 5 mM ascorbic acid, and 0.1% w/v BSA (Sigma, type V); the ratio of isolation medium to leaf tissue was 3:1 (v/w). The homogenate was filtered through six layers of nylon mesh and the plastids pelleted at 400 g for 2 min. The pellet was gently washed with isolation medium, then resuspended in 2.5 ml of hypotonic solution: 10 mM Tricine (pH 8.0), 10 mM MgCl2, 2 mM NaCl, and 2 mM ascorbic acid. Throughos rupture of the chloroplast envelopes was ensured by sonication for 5 s with a Branson Sonifier Cell Disrupter 200. Soluble enzymes were separated from chloroplast membranes by centrifugation at 45,000 g for 15 min.

To determine and correct for Chl of broken chloroplasts, the percentage of intact chloroplasts present after the initial centrifugation was determined by phase contrast microscopy. Chl was determined as described previously (2, 21).

Enzyme Activity. Chloroplast GPDHase activity was determined as described by Latzko and Gibbs (19) and Wolosiuk and Buchanan (24). The enzyme was activated by the addition of 0.5 ml of a solution containing 0.2 mM Tricine (pH 8.4) and 20 mM ATP to 0.1 ml of the solution containing the extracted enzymes. After 5 min of activation at 25°C, we added 0.75 ml of reaction medium (62.5 mM Tricine [pH 8.4], 16 mM MgCl2, 7.8 mM ATP, and 0.2 mM NADPH). The enzymic reaction was initiated by the addition of 0.1 ml of 50 mM glycerate 3-P and the oxidation of NADPH monitored at 340 nm.

FBPase activity was determined as described by Nishizawa et al. (11). The enzyme was activated by preincubation of 0.1 ml of enzyme extract with 0.35 ml of reaction medium (0.14 mM Tricine [pH 7.9], 14 mM MgCl2, and 7 mM DTT). After activating for 10 min at 25°C, the reaction was initiated by the addition of 0.01 ml of 60 mM fructose 1,6-bisphosphate. The reaction was run at 26°C, and terminated after 15 min by the addition of 0.5 ml of 10% (w/v) TCA. This solution was cleared by centrifugation for 10
min at 12,000 g in order to determine the inorganic Pi liberated colorimetrically (5).

RPKase activity was analyzed as described by Wolosiuk and Buchanan (25). The enzyme was activated by incubating 0.1 ml of the extract with 0.1 ml of 0.1 m Tricine (pH 7.6) and 15 mM DTT for 1 h at 25°C. After the activation was completed, 0.75 ml of the reaction medium was added. The reaction medium consisted of 66 mM Tricine (pH 7.6), 13 mM MgCl₂, 1.3 mM Pi, 2 units each of phosphoribioisomerase, pyruvate kinase, and lactate dehydrogenase. The reaction was initiated by the addition of 0.05 ml of 30 mM ribose-5-P and the oxidation of NADH monitored at 340 nm.

**Chemicals.** All compounds used were purchased from Sigma Chemical Company with the exception of the MgCl₂, CaCl₂, and NaCl (Mallinckrodt).

### RESULTS AND DISCUSSION

Determination of the influence of Fe nutrition on the activity of GPDHase, FBPase, and RPKase is complicated by the fact that these enzymes may be activated in vivo by products of light-generated reactions. A ferredoxin-thioredoxin activating system has been proposed (17) for the regulation of GPDHase (3, 24), RPKase (25), and FBPase (4, 5, 16). Alternatively, GPDHase and RPKase may be modulated by light-generated vicinal-dithiols (1, 7) and GPDHase has been reported to be activated by ATP or NADPH (9, 10, 15, 24). Thus, because these three enzymes require activation, it was necessary to add exogenous compounds to substitute for light activation in vivo. GPDHase was preincubated with ATP, and RPKase and FBPase with DTT to substitute for the ferredoxin-thioredoxin system (17) or the vicinal-dithiol system (1). These in vitro rates therefore represent the maximum rates obtainable with enzymes activated by nonphysiological means, and although they may reflect the amount of enzyme present, they do not indicate the extent of activation in vivo.

The influence of Fe nutrition on the three enzymes was investigated by withholding Fe nutritionally for 7 d to induce Fe deficiency chlorosis, then resupplying Fe to regreen the plants. After 1 week without Fe, leaf Chl content decreased from 49 ± 4.2 µg cm⁻² to 12 ± 2.4 µg cm⁻². Over the same period extractable activities of the enzymes on a per Chl basis increased substantially (Table I, Fig. 1). When expressed per unit area leaf tissue, however, the activities of the three enzymes under Fe stress were only slightly elevated over those of control plants (Fig. 2).

When Fe was resupplied, the Chl content of the previously Fe-deficient leaves rapidly increased, approaching control levels in 5 d. The per area activities of GPDHase and FBPase remained above control levels and changed little throughout the regreening process. RPKase activity differed in that the activity per area increased during Fe resupply, rather than remaining constant. The activities of all three enzymes decreased on a per Chl basis during Fe resupply, which reflected mainly the increase in Chl content.

These data show that although Chl content was reduced by 76% under Fe stress, the activities of the three enzymes were not negatively affected. Although there was some variation in the activity of the three enzymes in response to Fe stress and Fe resupply, the changes were generally much smaller than the changes in Chl content. Thus, these data, along with the previously reported data on RuBP carboxylase and lamellaren components (19, 23), support the conclusion that Fe stress influences photosynthesis via changes in the concentration of lamellar components rather than via changes in the enzymes of photosynthetic carbon metabolism. However, since Fe stress might influence the level of activation of the enzymes in vivo, we can infer only that Fe stress does not deplete the amount of enzyme present.

In reviewing the literature, it is evident that although some studies support the view that Fe stress preferentially affects lamellar components (e.g. Price and Carell [14]), others do not. Stocking (20) found that Fe stress reduced RuBP carboxylase activity as well as ferredoxin levels and lamellar content in corn. Perur et al. (12) reported an 82% reduction in chloroplast protein in iron deficient corn, whereas Shetty and Miller (18) observed a significant reduction in chloroplast protein (along with decreases in Chl and carotenoids) during Fe deficiency in tobacco. These more wide-ranging effects may be related to the duration and

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**Table 1. Rates of Chloroplast NADP-Glyceraldehyde-3-Phosphate Dehydrogenase, Chloroplast Fructose Bisphosphatase, and Ribulose 5-Phosphate Kinase Extracted from Control and Iron-Deficient Leaves**

<table>
<thead>
<tr>
<th></th>
<th>Control Plants</th>
<th>Iron-Deficient Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol h⁻¹ mg⁻¹ Chl</td>
<td>µmol h⁻¹ cm⁻²</td>
</tr>
<tr>
<td>GPDHase</td>
<td>250 ± 69</td>
<td>15.2 ± 3.6</td>
</tr>
<tr>
<td>FBPase</td>
<td>139 ± 51</td>
<td>10.5 ± 6.6</td>
</tr>
<tr>
<td>RPKase*</td>
<td>158 ± 24</td>
<td>7.9 ± 1.7</td>
</tr>
<tr>
<td>Chl (µg cm⁻²)</td>
<td>49.0 ± 4.2</td>
<td>12.0 ± 2.4</td>
</tr>
</tbody>
</table>

*Experiments performed with a different set of plants from those used for GPDHase and FBPase activities; growth conditions were identical for both groups.
in different light/CO₂ environments.

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LITERATURE CITED