Changing Activity of Glucose-6-Phosphate Dehydrogenase from Pea Chloroplasts during Photosynthetic Induction

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ABSTRACT

Light inactivation of glucose 6-phosphate dehydrogenase is rapid and occurs before photosynthetic O₂ evolution is measurable in intact chloroplasts. Likewise, dark activation is rapid. The major light induced change in the kinetic parameters of glucose 6-phosphate dehydrogenase is in maximal velocity.

The first enzyme of the oxidative pentose phosphate pathway, glucose 6-P dehydrogenase (EC 1.1.1.49), is inactivated when pea chloroplasts are irradiated (3). This light inactivation probably prevents operation of a futile cycle involving glucose 6-P, NADPH, and oxidative and reductive pentose phosphate pathway enzymes. We have now examined the kinetics of light inactivation of glucose 6-P dehydrogenase in intact chloroplasts during photosynthetic induction and the kinetic parameters of the active (dark) and less active (light) forms of the enzyme.

Fickenscher and Scheibe (9) have partially purified cytosolic glucose 6-P dehydrogenase from peas. Unlike the purified chloroplastic enzyme (20), the cytosolic enzyme was not DTT or DTT/thioredoxin sensitive. On the basis of this lack of sensitivity to DTT and the results of antibody experiments with extracts from leaves which had been illuminated, Fickenscher and Scheibe concluded that the cytosolic enzyme was not light inactivated. But in their antibody experiments the error in activity determinations for the cytosolic enzyme from darkened and illuminated leaves was 18 and 23%, respectively (Fig. 3 in Ref. 9). Since errors are additive, the error in the determination of light inactivation was then the same as the expected change in activity if the cytosolic enzyme were 40% inactivated by light. Because previous work in this laboratory (4-6) indicated that the cytosolic enzyme was light and DTT inactivated, we have reinvestigated the DTT-dependent inactivation of the enzyme in whole leaf extracts.

MATERIALS AND METHODS

Photosynthetic O₂ Evolution and Determination of Kinetic Parameters. Chloroplasts were isolated from pea (Pisum sativum L., var Little Marvel) seedlings and photosynthetic O₂ evolution assayed as described by Marques and Anderson (15) except that the chloroplast concentration was increased. Where activity with time of illumination was followed, the chloroplast content in the O₂ evolution assay was equivalent to 250 to 280 µg Chl ml⁻¹. Where the changes in kinetic parameters were investigated, the chloroplast content during illumination was equivalent to 400 to 600 µg Chl ml⁻¹.

Glucose 6-P dehydrogenase activity was assayed spectrophotometrically. Change in A₅₄₀ was followed on a Varian Cary 210 or 219 recording spectrophotometer.

When light inactivation and dark reactivation were measured in the same experiment as photosynthetic O₂ evolution, 10 µl aliquots (2.5-2.8 µg Chl) were removed from the O₂ evolution assay mixture directly into the assay cuvette, which contained 2.5 µM glucose 6-P (Na⁺), 0.2 mM NADP, 50 mM Hepes (K⁺), 10 mM KCl, 5 mM MgCl₂, 1 mM EDTA (pH 7.8), and Triton X-100 (0.03%) (final volume, 1 ml). The assay temperature was 24°C.

When the kinetic parameters were determined, an aliquot (1.5 ml) was removed from the O₂ evolution assay mixture after 5 min incubation in the dark or 10 min incubation in the light, diluted to 15 ml with 50 mM Hepes (K⁺), 10 mM KCl, 5 mM MgCl₂, 1 mM EDTA (pH 7.8), and centrifuged (27,000g, 15 min). Glucose 6-P dehydrogenase was partially reactivated during this procedure. After centrifugation aliquots (100 µl, equivalent to 4-6 µg Chl) of the supernate were used for the estimation of kinetic parameters. The components of the assay were as above except for the varied substrate and that Triton X-100 was omitted. Glucose 6-P was varied from 0.03 to 4 mM by even reciprocal intervals for the Kₘ (glucose 6-P) estimation. NADP was varied from 0.2 to 2 µM by even reciprocal intervals for the Kₘ (NADP) estimation. Kₘ and Vₘₐₓ values were estimated from steady state velocities with the aid of the IBM 3081K64 computer at the University of Illinois Chicago Computer Center and the program of Hanson et al. (10). The reciprocal of the variance was used as a weighting factor for estimation of mean Kₘ and SE.

For determination of the effect of pH and Mg²⁺ on the kinetics of the dark form of the enzyme, Tris (Cl⁻) (100 mM) replaced Hepes (K⁺) in the assay and KCl, EDTA, and Triton X-100 were omitted. Chloroplasts were lysed in the assay buffer, which included MgCl₂ when the pH was 7.8. The extract was centrifuged (27,000g, 20 min) to remove membranes and the supernatant solution was used as a source of enzyme.

Experiments with Whole Leaf Extracts. Pea plants (10-12 d old) were placed in a dark cabinet overnight. In the morning 10 leaves were cut from the plants, rinsed in buffer, and homogenized with a glass tissue grinder in 4 ml of 100 mM Tris (Cl⁻) (pH 7.8) containing Triton X-100 (0.1%) in darkness. The homogenate was centrifuged (27,000g, 5 min) and the supernatant treated with DTT. In these experiments the Hepes (K⁺), KCl, EDTA, MgCl₂ buffer used in the assay in the experiments with chloroplast extracts was replaced with 100 mM Tris (Cl⁻) (pH 7.8).

Protein was estimated by the method of Bradford (8).
RESULTS AND DISCUSSION

Inactivation of Glucose 6-P Dehydrogenase in Intact Chloroplasts. Light inactivation of glucose 6-P dehydrogenase in intact pea chloroplasts is very rapid and occurs before photosynthetic O₂ evolution is detected (Fig. 1). Activation of reductive pentose phosphate cycle enzymes occurs at a comparable rate in this system (16). Huber (13) has suggested that glucose 6-P dehydrogenase activity might contribute to the buildup of intermediates during induction. However, in experiments which are comparable to the experiments reported here, we measured metabolite levels and found that glyceralte 3-P, triose-P, hexose 1.6-bisP, sedoheptulose 1.7-bisP and ribulose 1.5-bisP levels remained low and apparently constant during the first 2 min of illumination (16). In the present experiments the dehydrogenase was almost completely inactivated during the first 30 s of illumination. We conclude that it is not likely that breakdown of glucose 6-P by the dehydrogenase during light inactivation contributes substantially to the chloroplast metabolite pool.

Dark activation of glucose 6-P dehydrogenase is also very rapid (Fig. 1). If levels of gluconate 6-P increase in proportion to glucose 6-P dehydrogenase activity, then gluconate 6-P inhibition of rubulose 5-P kinase (1) and ribulose bisP carboxylase (21) probably helps to prevent operation of the reductive pentose phosphate pathway in the dark.

Metabolically the most significant effect of light inactivation of stromal glucose 6-P dehydrogenase is probably the decrease in maximal velocity (Fig. 1; Table I). (This follows from a consideration of the Michaelis-Menten equation. A change in maximal velocity always results in a directly proportional change in \( v \), but a change in \( K_m \) results in no change in \( v \) when \( S \) is high and an inversely proportional change in \( v \) only when \( S \) is very low.) The \( K_m \) value for glucose 6-P decreases with the dark/light transition, but the change is less than an order of magnitude and may simply reflect the change in conformation of the enzyme (20). The difference in the \( K_m \) (NADP) values is small and only slightly larger than the summed error in the determinations. It seems unlikely that this change affects metabolism. Conversely, the most significant effect of dark modulation is most likely the increase in maximal velocity. The \( K_m \) values reported here for the enzyme is crude stromal extracts are comparable to those reported for the purified enzyme (20). In earlier experiments, under quite different conditions, we found no significant change in the apparent affinity for glucose 6-P when the dehydrogenase was light inactivated (4). In contrast light activation affects both maximal velocity and \( K_m \) in the case of the reductive pentose phosphate cycle enzyme fructose bisphosphatase (15) and cooperativity in the case of NADP-linked glyceraldehyde 3-P dehydrogenase (14).

At pH 7.2, and in the absence of added Mg²⁺, conditions which may approximate the stroma in the dark chloroplast (11), the kinetics become sigmoidal when glucose 6-P is the varied substrate (Fig. 2). Non-Michaelian kinetics have been reported for the enzyme from several plant species (7, 12, 17, 18). Apparently even in the dark the dehydrogenase will not be active unless glucose 6-P concentrations exceed a threshold level. Because P-glucose isomerase has Michaelis-Menten kinetics (\( K_m \) for glucose 6-P is about 0.2 mM, LE Anderson, MJ Hansen, unpublished data), glycolysis will be favored in the dark when hexose monoP levels are low.

**DTT Inactivation of Glucose 6-P Dehydrogenase in Whole Leaf Extracts.** Clearly most of the enzyme in the whole leaf extract, which is probably at least 50% cytosolic (6, 9), is DTT inactivated (Fig. 3). In experiments in which the leaves were chopped with razor blades and the debris, whole cells, and

![Fig. 1. Glucose 6-P dehydrogenase activity (data points) and rate of CO₂-dependent O₂ evolution (solid line without data points) from isolated chloroplasts after dark/light and light/dark transition at 25°C. Light off and on as indicated by dark or light bar at top of figure. Similar results were found in two additional experiments.](image)

![Fig. 2. Effect of glucose 6-P levels on the activity of the dark form of chloroplastic glucose-6-P dehydrogenase at pH 7.8 (O) and pH 7.2 (■). The assay media was 100 mM Tris (Cl⁻), 0.2 mM NADP, and glucose 6-P at the indicated concentrations. MgCl₂ (5 mM) was included in the pH 7.8 assay. The protein concentration in the cuvette was 650 \( \mu \)g ml⁻¹. The n value was 1 when the pH was 7.8, and 2 when the pH was 7.2. Similar results were obtained in a second experiment.](image)

Table I. \( K_m \) Values of Glucose 6-Phosphate Dehydrogenase from Pea Chloroplasts Before and After Light Inactivation

<table>
<thead>
<tr>
<th>Varied Substrate</th>
<th>Dark Form</th>
<th>Light Form</th>
</tr>
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<tbody>
<tr>
<td>NADP</td>
<td>0.9 ± 0.1 (2)*</td>
<td>1.3 ± 0.2 (2)</td>
</tr>
<tr>
<td>Glucose-6P</td>
<td>340 ± 20 (3)</td>
<td>140 ± 10 (3)'</td>
</tr>
</tbody>
</table>

* Number in parentheses is number of separate determinations. * If the substrate for the chloroplast enzyme is the \( \beta \) anomer as it is for glucose 6-P dehydrogenases (19), the \( K_m \) (glucose 6-P) values are 220 \( \mu \)M for the dark form and 87 \( \mu \)M for the light form of the enzyme. * Note that this value is an average value in these experiments. The real value at any time will be a function of the light treatment of the chloroplasts.
were denaturation enzyme seems likely by extracts was phoretically and about likewise (data from above as shown). Consistent with our previous results, the present results indicate that native cytosolic glucose 6-P dehydrogenase from pea leaves is DTT inactivated. Experiments in which intact plants were irradiated were less definitive. There was about 25% inactivation of the enzyme in whole leaves and in the supernatant after leaves were chopped with razor blades, as above (data not shown). In earlier experiments (6) the chloroplastic and cytosolic forms of the enzyme separated electrophoretically were both apparently inactivated when seedlings were irradiated. Notably, the dehydrogenase in crude whole leaf extracts was quite stable (Fig. 3): Fickenscher and Scheibe (9) reported a rapid loss of activity after breaking the leaf cells. It seems likely that the cytosolic glucose 6-P dehydrogenase purified by Fickenscher and Scheibe (9), like our first preparation of the chloroplastic enzyme (2), lost sensitivity to DTT as a result of partial denaturation during isolation.

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