Light Modulation of Glucose-6-Phosphate Dehydrogenase

PARTIAL CHARACTERIZATION OF THE LIGHT INACTIVATION SYSTEM AND ITS EFFECTS ON THE PROPERTIES OF THE CHLOROPLASTIC AND CYTOPLASMIC FORMS OF THE ENZYME

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ABSTRACT

Light inactivation of glucose-6-phosphate dehydrogenase within the pea (Pisum sativum L.) leaf chloroplast has a narrow pH optimum between 7.2 and 7.4 and is NADPH-sensitive. The pH optimum for dark activation is slightly lower. Inactivation apparently results in a simple decrease in maximal velocity of the chloroplastic and cytoplasmic forms of the enzyme with no concomitant change in pH optimum or \( K_m \) (glucose-6-phosphate).

The chloroplastic form of glucose-6-P dehydrogenase (EC 1.1.1.49) is inactivated by a dithiol-containing light effect mediator which is associated with the photosynthetic electron transport system (3) and inhibited by NADPH (13, 20). The purpose of the present experiments was to examine the role of these and other types of modulation in the control of the activity of this enzyme and hence of the potential operation of the oxidative pentose phosphate pathway within the chloroplast.

MATERIALS AND METHODS

Plant Material. Pea (Pisum sativum L., var. Little Marvel) plants were grown in a soil-perlite mixture under natural light for 12 to 15 days in a greenhouse.

Preparation of Extracts and Chloroplasts. Whole shoot extracts were prepared by grinding green shoots in 20 mm, pH 7.4, tris-HCl with a mortar and pestle, filtering through four layers of cheesecloth, and centrifuging for 15 min at 12,000g. Chloroplasts and cytoplasmic fractions were prepared essentially as described previously (2) except that in some cases (specified) the isotonie medium of Stokes and Walker (18) was used. Stromal and particulate fractions of broken chloroplasts were prepared as described previously (3) except that for determination of pH optima of light inactivation and dark activation the concentration of HEPES in the resuspension medium was only 5 mm and in all cases MgCl\(_2\) was only 2 mm.

Dark treatment consisted of placing plants in darkness overnight. Unless otherwise noted subsequent manipulation of dark-treated material was in darkness or very dim light. For conditions of light treatment of whole plants see individual experiments.

Enzyme Assays. Glucose-6-P dehydrogenase and NADP-linked glyceraldehyde-3-P dehydrogenase in NADP-generating direction were assayed as previously described (7).

Determination of pH Optima. Assay mixture pH was measured at room temperature with a Radiometer pH meter 26 after activity of the enzyme or system was determined.

Determination of Kinetic Constants. The activity of a constant amount of the specified enzyme was measured at six different concentrations of substrate. Each set of reaction rates and corresponding substrate concentrations was analyzed using the program of Hanson et al. (9) and the IBM 370 computer at the University of Illinois, Chicago Circle Computer Center.

Light Inactivation. Light inactivation of glucose-6-P dehydrogenase in the broken chloroplast system was assayed as described previously except that the light source (a General Electric 300 W, 120 v cool beam flood lamp [No. 300 Par 56/WFL]) was 35 cm distant. Light intensity was 6000 ft-c. Initial velocity of light modulation (inactivation) or dark modulation (activation) is expressed in nmol product formed min\(^{-2}\) mg stromal protein\(^{-1}\) (\( v_o (\text{mod}) \)) units, i.e. change in enzyme activity per unit time.

Protein and Chlorophyll Estimation. Protein was estimated by Biuret method after precipitation with acetone (2) and Chl by the method of Arnon (5).

Chemicals. Biochemicals were the product of Sigma Chemical Co. Other chemicals were analytical reagent grade. Pea seeds were obtained from Northrup and King Seed Company, Chicago.

RESULTS AND DISCUSSION

The chloroplastic form of glucose-6-P dehydrogenase can be separated from the cytoplasmic form by electrophoresis (4) or by ammonium sulfate chromatography (16) and, of course, by separation of chloroplasts from cytosol, which indicates that there probably are both positional and structural differences in the two forms of the enzyme in higher plants. Light treatment of intact pea plants does not change the pH optima of either form of the enzyme nor does DTT\(^3\) treatment of the crude chloroplastic or cytoplasmic extract affect the shape of the pH dependence curve (Fig. 1, A and B). In the present experiments we did not attempt to eliminate or correct for 6-P-gluconate dehydrogenase activity, but rather assumed that since its pH dependence curve is similar to that for the sugar-P dehydrogenase (16) that it would act essentially as a secondary coupling enzyme, except that differences would be disproportionately magnified. Levels of activity of the two dehydrogenases within the chloroplasts are about the same.

We have also examined the effect of light treatment of the intact plant and DTT treatment of extracts on the pH optima of the NADP-linked chloroplastic form of glyceraldehyde-3-P dehydrogenase (Fig. 2). As with glucose-6-P dehydrogenase light treatment did not appreciably affect the optimum. In contrast DTT treatment of the extract does shift the optimum toward a higher pH (Fig. 2).

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\(^3\) Abbreviations: DTT: dithiothreitol; DSPD: dicyclohexylidene propane-3,6-diamine; EM: electron micrograph.
more alkaline pH. Similar results were obtained for purified spinach leaf fructose-1, 6-diP phosphatase with DTT treatment by Baier and Latzko (6). Light treatment alone does not markedly affect the optimal pH of either dehydrogenase. The nonphysiological and probably less specific effector DTT affects the pH optimum for both NADP-linked glyceraldehyde-3-P dehydrogenase and fructose-1,6-diphosphatase, but there is no reason to believe that this effect is necessarily related to the effect of light on the activity of either enzyme. Light and DTT treatment affect the maximal velocity values of both forms of glucose-6-P dehydrogenase, when glucose-6-P is the variable substrate, and of glyceraldehyde-3-P dehydrogenase when NADPH is the variable substrate (Table I). Our glyceraldehyde-3-P dehydrogenase results are consistent with those of Melandri et al. (15). The effect elicited by light and DTT treatment is similar in each of these cases, although the DTT effect is more pronounced. Light or DTT treatment results in apparent noncompetitive inhibition, with respect to glucose-6-P, of glycerol-dehydrogenase and in noncompetitive stimulation, with respect to NADPH, of NADP-linked glyceraldehyde-3-P dehydrogenase. Neither light nor DTT significantly affects the apparent affinity for the variable substrates in the present experiments. At the molecular level, this type of modulation is consistent either with total activation or inactivation of the affected enzyme (i.e. classical noncompetitive inhibition or stimulation) or with allosteric modification of the activity of the enzyme through an effect on a modulator site not identical with the active site.

In crude chloroplast extracts glucose-6-P dehydrogenase is inactivated by a membrane-bound light effect mediator which is associated with the electron transport system (3). Light inactivation is reversible in this system; preliminary experiments indicate that separate systems are involved in light and dark modulation of enzyme activity (L. E. Anderson, unpublished data). Time
Table 1. Effect of Light, Dark, and DTT Treatment on Michaelis Constants and Maximal Velocity Values of Pea Leaf Chloroplastic and Cytoplasmic Glucose-6-P Dehydrogenase and NADP-linked Glyceraldehyde-3-P Dehydrogenase

For the chloroplastic glucose-6-P dehydrogenase experiments chloroplasts, prepared from plants held overnight in the dark, were suspended in 50 mM potassium HEPES, 5 mM MgCl₂, 1 mM EDTA, pH 7.2, and were either lysed immediately by addition of 9 vol cold deionized water (dark-treated) or were irradiated at ice bath temperatures for 2 min with 725 ft-c white light and then lysed. DTT-treatment consisted of making extract of dark-treated chloroplasts 50 mM in DTT and allowing the extract to remain on ice for at least 30 min. Membrane fragments were removed by centrifuging for 10 min at 27,000g and the supernatant solution was used in assays. Cuvettes contained 100 μmol tris-HCl, pH 7.8, 0.2 μmol NADP, 0.12, 0.148, 0.195, 0.283, 0.517, or 3 μmol glucose-6-P, and extract in a total volume of 1 ml. For the cytoplasmic glucose-6-P dehydrogenase experiments, cytoplasmic extract was prepared from whole plants which were kept overnight in the dark (dark-treated) or which were (after the dark treatment) exposed to 550 ft-c white light for 30 min at room temperature. The isotonic media of Stokes and Walker was used in these experiments. DTT treatment consisted of making the cytosol extract from dark-treated plants 50 mM in DTT and allowing the extract to stand for at least 30 min at ice bath temperatures. Assay conditions as for chloroplastic glucose-6-P dehydrogenase. For the NADP-linked glyceraldehyde-3-P dehydrogenase experiments, extracts were prepared by grinding light- or dark-treated pea plants (see cytoplasmic glucose-6-P dehydrogenase experiments) in 20 mM, pH 7.4, tris-HCl. Cuvettes contained 5 μmol 3-P-glyceric acid, 10 μmol MgCl₂, 2.5 μmol ATP, 100 μmol tris-HCl, pH 7.8, extract, and 10, 21.4 16.2, 23.6, 43.1, or 250 μmol NADPH in a total volume of 1 ml. No exogenous P-glycerate kinase was added.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Variable Substrate</th>
<th>Experiment</th>
<th>Dark</th>
<th>Light</th>
<th>DTT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vmax (μmoles product formed min⁻¹ mg⁻¹ protein⁻¹)</td>
<td>Km (mM)</td>
<td>Vmax (μmoles product formed min⁻¹ mg⁻¹ protein⁻¹)</td>
</tr>
<tr>
<td>Chlo-</td>
<td>Glucose-6-P dehydro-</td>
<td>I</td>
<td>0.56 ± 0.08</td>
<td>34 ± 1</td>
<td>0.56 ± 0.04</td>
</tr>
<tr>
<td>plast-6-P</td>
<td>genase</td>
<td>II</td>
<td>0.50 ± 0.05</td>
<td>29 ± 1</td>
<td>0.48 ± 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III</td>
<td>0.33 ± 0.06</td>
<td>34 ± 4</td>
<td>0.47 ± 0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IV</td>
<td>0.51 ± 0.04</td>
<td>34 ± 4</td>
<td>0.47 ± 0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>V</td>
<td>0.32 ± 0.02</td>
<td>34 ± 1</td>
<td>0.29 ± 0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VI</td>
<td>0.71 ± 0.01</td>
<td>81 ± 7</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VII</td>
<td>0.9 ± 0.1</td>
<td>56 ± 3</td>
<td>0.56 ± 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VIII</td>
<td>0.37 ± 0.06</td>
<td>45 ± 3</td>
<td>0.32 ± 0.05</td>
</tr>
<tr>
<td>NADP-</td>
<td>NADPH</td>
<td>IX</td>
<td>0.05 ± 0.01</td>
<td>460 ± 20</td>
<td>0.083 ± 0.008</td>
</tr>
<tr>
<td>linked</td>
<td></td>
<td>X</td>
<td>0.031 ± 0.003</td>
<td>270 ± 15</td>
<td>0.027 ± 0.004</td>
</tr>
<tr>
<td>Glyceraldehyde-3-P</td>
<td>dehydrogenase</td>
<td>XI</td>
<td>0.024 ± 0.006</td>
<td>80 ± 10</td>
<td>0.041 ± 0.004</td>
</tr>
</tbody>
</table>

The course of dark activation of glucose-6-P dehydrogenase is shown in Figure 3.

It is possible to determine pH optima both for the LEM system and for dark activation (Fig. 4). There is a sharp optimum for light inactivation at pH 7.3 and sharp optimum for dark reactivation at pH 7.1. The curves overlap with inactivation being favored at higher pH values and activation being favored at lower pH values. Similar curves for pH dependence have been obtained for light modulation (activation) of the activity of ribulose-5-P kinase and fructose-1,6-diP phosphatase (J. Duggan and H-M. Chin, unpublished data).

The changes in, and the effect of light intensity on, stromal pH have been estimated by Heldt et al. (11, 19). The pH of the stroma is about 7.1 in dark and 8.1 in the fully illuminated chloroplast. Our experiments indicate that light inactivation and dark activation will occur optimally as the pH is shifted from the low value in the stroma of the nonilluminated chloroplast to the higher pH of the stroma of the fully illuminated chloroplast. At pH 8.1 (fully illuminated chloroplast) the enzyme will have been inactivated; at pH 7.1 (nonilluminated chloroplast) the enzyme will be activated. The LEM system will actively modulate activity of the enzyme only at pH values between 7.1 and 7.5, or, in other words, either in chloroplasts which are not fully illuminated, or in chloroplasts which have just been illuminated and in which stromal pH is still rising. Thus the light and dark modulator systems seem to have been designed to act as on and off switches for the operation of this enzyme and hence of the oxidative pentose phosphate pathway within the chloroplast.

Since the LEM and NADP apparently both can act as electron acceptors in photosynthetic electron transport, it may well be that NADP competes with the LEM for electrons and hence inhibits the inactivation of glucose-6-P dehydrogenase. That this is true is illustrated with a reciprocal drop in NADP (10,

Fig. 3. Reactivation of glucose-6-P dehydrogenase in broken chloroplast system in darkness. Glucose-6-P dehydrogenase activity is plotted versus time after light is turned off. Chl concentration, 13.5 μg ml⁻¹; stromal protein concentration, 420 μg ml⁻¹. Time in light, 3 min. Reactivation stopped by 10-fold dilution of the reactivation assay mixture in ice-cold deionized H₂O and glucose-6-P dehydrogenase activity assayed in triplicate on each sample.
Fig. 4. Effect of pH on the inactivation of glucose-6-P dehydrogenase by light (O—O) and reactivation in dark (■—■) in broken chloroplast system. Data shown is a composite of three experiments for light inactivation and two for dark reactivation. Initial velocity of modulation relative to maximal velocity of modulation in each separate experiment was plotted versus pH. Values were normalized and replotted to obtain curves shown here. Activity of glucose-6-P dehydrogenase in stromal extract used in light inactivation was 8.15, and 22 nmol NADPH formed min⁻¹ mg protein⁻¹ t₀, stromal protein concentration was 4.1, 2.8, and 2.9 mg ml⁻¹, respectively, in modulation experiment and maximal initial velocity modulation was 11.23, and 51 vₑ (mod) units. Activity of glucose-6-P dehydrogenase in stromal extract used in dark reactivation experiments was 8.6 and 8 nmol NADPH formed min⁻¹ mg protein⁻¹ at t₀ (by extrapolation), stromal protein concentration was 4.5 and 8 mg ml⁻¹ and maximal initial velocity modulation was 5.6 and 7.2 vₑ (mod) units. Light exposure prior to dark reactivation was 2 min. Chl concentration was about 100 µg ml⁻¹ (constant within experiments). Light modulation of the activity of glucose-6-P dehydrogenase is clearly pH-dependent.

Table II. Effect of NADP and NAD on Light Inactivation of Glucose-6-P Dehydrogenase in Broken Chloroplast System

<table>
<thead>
<tr>
<th>NADP</th>
<th>NAD</th>
<th>Glucose-6-P Dehydrogenase before Illumination</th>
<th>Initial Velocity of Light Modulation</th>
<th>Stromal Protein Conc during Illumination</th>
</tr>
</thead>
<tbody>
<tr>
<td>15%</td>
<td>0%</td>
<td>mmol NADPH formed min⁻¹ mg stromal⁻¹</td>
<td>mg ml⁻¹</td>
<td></td>
</tr>
<tr>
<td>77</td>
<td>65</td>
<td>10.2</td>
<td>11</td>
<td>2.3</td>
</tr>
<tr>
<td>57</td>
<td>43</td>
<td>8.3</td>
<td>10.8</td>
<td>2.0</td>
</tr>
<tr>
<td>68</td>
<td></td>
<td>8.9</td>
<td>13.8</td>
<td>2.8</td>
</tr>
</tbody>
</table>

13. Within the intact leaf, where shuttle system(s) (12, 17) for reduced pyridine nucleotide can operate, this burst is apparently quickly compensated for and NADPH and NADP levels within the chloroplast rapidly (minutes) return to “normal” (see Fig. 1, ref. 19). Immediately following illumination, then, it seems very likely that free NADP present in the stroma will be reduced, in preference to the LEA, after which the LEA will be reduced and hence capable of inactivating glucose-6-P dehydrogenase. That such a competition can and does occur in the intact chloroplast is evidenced by the enhancement by DSPD of inactivation in the intact chloroplast (3). Unfortunately in the present experiments a general inhibition of the LEA system for glucose-6-P dehydrogenase by triphosphopyridine nucleotides cannot be ruled out, since NADPH inactivates glucose-6-P dehydrogenase directly (see below) making it impossible to test the effect of the reduced nucleotide in the light inactivation system. As would be anticipated NAD also acts as an inhibitor of the LEA system (Table III). At 6 mM concentrations ATP inhibits (about 25%, data not shown), ADP and AMP do not.

Inhibition of spinach chloroplast glucose-6-P dehydrogenase by NADPH and by ribulose-1,5-diphosphate by Lendzian and Bassham (13). The pea leaf enzymes apparently are not inhibited by ribulose-1,5-diphosphate (4) although both forms of the enzyme are sensitive to NADP (data not shown). Lendzian and Bassham (13) found that NADPH was competitive with respect to NADP and suggested that the regulation of the enzyme by light might be mediated by NADPH/NADP ratios and by ribulose-1,5-diphosphate. It is not clear how they explain the inactivation of glucose-6-P dehydrogenase in intact plants observed by Lendzian and Ziegler (14) as well as by us (4), which for true competitive inhibition should not persist after electrophoresis or when the cell or chloroplast is disrupted and the inhibitor is diluted. Wildner (20), like Lendzian and Bassham, has studied inhibition of chloroplastic glucose-6-P dehydrogenase by NADPH and has suggested that the enzyme is controlled by “reduction charge.” His experiments were done with shocked pea leaf chloroplasts at pH 8. The conditions probably correspond in some respects to the in vivo situation in the fully illuminated chloroplast. We agree with Wildner and with Lendzian and Bassham that NADPH/NADP ratios probably do control the activity of the enzyme at pH 8 in the illuminated chloroplast. However, since (a) arsenite interferes with light inactivation of glucose-6-P dehydrogenase but not with NADP reduction (see ref. 3), (b) there is no evidence for the involvement of soluble factors in light inactivation (3), and (c) light inactivation occurs in broken chloroplast preparations where the stromal fraction is necessarily dilute and hence NADPH levels probably much lower than in the intact chloroplast, it is obvious that light inactivation is not simply the result of inhibition of glucose-6-P dehydrogenase by NADPH. Rather it seems more likely that glucose-6-P dehydrogenase may be inhibited within the chloroplast by the LEA and inhibited by NADPH. It is interesting to note that the enzyme from Anacystis nidulans is inactivated by light (7) and, like the higher plant chloroplastic enzyme, sensitive to NADPH (8).

The inactivation and inhibition of the activity of both chloroplastic and cytoplasmic forms of glucose-6-P dehydrogenase within the pea leaf is apparently a highly integrated and complex process. On the basis of the data presented here and by inference from other studies (stromal pH changes reported by Heldt et al. [11]; changes in NADPH to NADP levels reported by Heber and Santarius [10] and by Lendzian and Bassham [13]; changes in metabolite levels reported by Bassham’s group as well as by Latzko and Gibbs [see ref. 1]), we can postulate that the sequence of events in Figure 5 or some similar sequence may occur when a green pea leaf is illuminated. The immediate response of chloroplastic glucose-6-P dehydrogenase to light will be a decrease in activity as a result of inhibition by NADPH produced when the leaf is illuminated. The second and slower response will be inactivation by the light effect mediator system. Both processes will be important in controlling the activity of this

![Figure 5](https://example.com/figure5.png)

**Fig. 5.** Possible sequence of events leading to inactivation of glucose-6-P dehydrogenase in pea leaf chloroplast in light and to reactivation in darkness. ■, active form of glucose-6-P dehydrogenase; ○, inactive form; shading indicates inhibition by NADPH.
enzyme. Clearly, the regulation of this enzyme is highly complex and involves interaction of a number of different factors and systems within the chloroplast. The one factor most important in controlling the activity of this enzyme at any single point in time will depend on environmental conditions external to the plant, and on internal microenvironmental factors as well, including most likely even the position and hence light exposure of the individual chloroplast within the cell.

Acknowledgments—The effect of light on pH dependence of the pea leaf glucose-6-P dehydrogenases and some of the Km and Vmax determinations for this enzyme are from experiments conducted by K. E. Y. Park, whom we thank for allowing us to use her data. We also thank J. McCorkle, L. Sykora, and staff at the University of Illinois, Chicago Circle Greenhouse, for growing the pea plants used in these experiments.

LITERATURE CITED