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Light Modulation of Phosphofructokinase in Pea Leaf Chloroplasts[1]

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ABSTRACT

Chloroplastic phosphofructokinase, phosphorylase, phosphoglucomutase, and phosphoglucoisomerase in peas are light inactivated. The effect of light on phosphofructokinase is mimicked by dithiothreitol. DCMU, arsenite, and sulfite inhibit light modulation of the enzyme. No effect of light inactivation on the K_m (fructose-6-P) or pH optima of phosphofructokinase was observed.

Phosphofructokinase (ATP:D-fructose-6-P 1-phosphotransferase, EC 2.7.1.11) is one of the key enzymes of glycolysis. It is an important regulatory enzyme in animals (26), plants (10), and bacteria (7). Metabolites such as P-enolpyruvate (17–20), p-glycollate (15, 17, 20), citrate (12, 17), and ATP (12, 16) affect the activity of this enzyme. It has previously been shown that pea leaf P-fructokinase is light inactivated (13). We now report that the chloroplastic form of this enzyme in peas is light inactivated. As with most light modulated enzymes, DTT-treatment mimics the effect of light. We also report that three other enzymes which are involved in the breakdown of starch, phosphorylase (EC 2.4.1.1), P-glucomutase (EC 2.7.5.1) and P-glucoisomerase (EC 5.3.1.9), are light inactivated.

MATERIALS AND METHODS

Preparation of Chloroplasts. Chloroplasts were prepared from 9- to 11-d-old pea (*Pisum sativum* L., Little Marvel) plants according to the method of Cockburn *et al.* (9). Broken chloroplasts were prepared by suspending the chloroplast pellet in at least 10 volumes of 50 mM Hepes (K^+), 10 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA (Na^+) (pH 7.5).

Light Inactivation. Light inactivation of P-fructokinase in broken chloroplasts was assayed as described previously (3) except that the light intensity was 5,500 ft-c. $MgCl_2$ concentration was 5 mM. For light inactivation of phosphorylase, P-glucomutase and P-glucoisomerase, intact chloroplasts in resuspension media (0.33 M sorbitol, 2 mM $MgCl_2$, 2 mM EDTA, 50 mM Hepes (K^+) [pH 7.2], 25), were incubated at room temperature in the dark for 10 min. An aliquot was removed and lysed by 10-fold dilution in cold distilled H_2O. The remaining chloroplasts were irradiated for 10 min after which the chloroplasts were lysed as above. The lysed chloroplast preparations were centrifuged for 10 min at 12,000*g* and the activity in the supernatant measured.

Enzyme Assays. Phosphofructokinase was assayed according to the method of Kelly and Latzko (15). In the experiments reported here the reaction was initiated by the addition of ATP. Change in A_{340} was followed using a Cary 210 or 219 recording spectrophotometer. A cuvette containing all of the components of the assay mixture except ATP was placed in the reference beam.

Phosphorylase was assayed by the method of Matheson and Richardson (22) except that 0.1% maltodextrin was used as substrate, pH was 7, and EDTA was omitted. P-glucomutase was assayed as described by Mühlbach and Schnarrenberger (23) except that the buffer was Tris-HCl. P-glucoisomerase was assayed with fructose-6-P as substrate as described by Schnarrenberger and Oeser (24). Change at A_{340} was followed using a Cary 219 or Gilford 2400 recording spectrophotometer.

All assays were conducted at room temperature (about 22°C).

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

Determination of Kinetic Constants. The P-fructokinase activity of a constant amount of chloroplast extract was measured at 0.05, 0.1, 0.2, 0.25, 0.5, 0.75, and 1 mM concentrations of fructose-6-P. The data were analyzed using the program of Hanson *et al.* (12) and the IBM 370 computer at the University of Illinois, Chicago Circle Computer Center.

Protein and Chl Content. Protein content was estimated by biuret method (21) and Chl by the method of Arnon (6).

Chemicals. Biochemicals were obtained from Sigma. Pea seeds were obtained from Northrup and King Seed Company, Chicago.

RESULTS AND DISCUSSION

Like other important regulatory enzymes in the chloroplast, P-fructokinase is light modulated. The time course for light inactivation is shown in Figure 1. Almost 70% of the initial dark activity was lost when a broken chloroplast preparation was exposed to light for 2 min. It can be seen that the inactivation is reversible. When the preparation was transferred back to darkness, the initial activity was fully restored (Fig. 1). Similar results were obtained with intact chloroplasts. Light modulation of the enzyme was imitated by DTT treatment. In a typical experiment activity of 4.9 nmol fructosebisphosphate formed min^{-1} mg $protein^{-1}$ was decreased 57% by exposing a broken chloroplast preparation to 50 mM DTT for 2 h at 0°C . Light and DTT have previously been shown to inactivate glucose-6-P dehydrogenase and to activate a number of enzymes of photosynthetic carbon metabolism in peas and other plants (1).

DCMU inhibited light inactivation of P-fructokinase (Fig. 1, legend), which indicates that the photosynthetic electron transport system is involved. Arsenite and sulfite, likewise, inhibit light inactivation of the enzyme (Fig. 1, legend). Both DCMU and arsenite would be expected to affect light modulation by either

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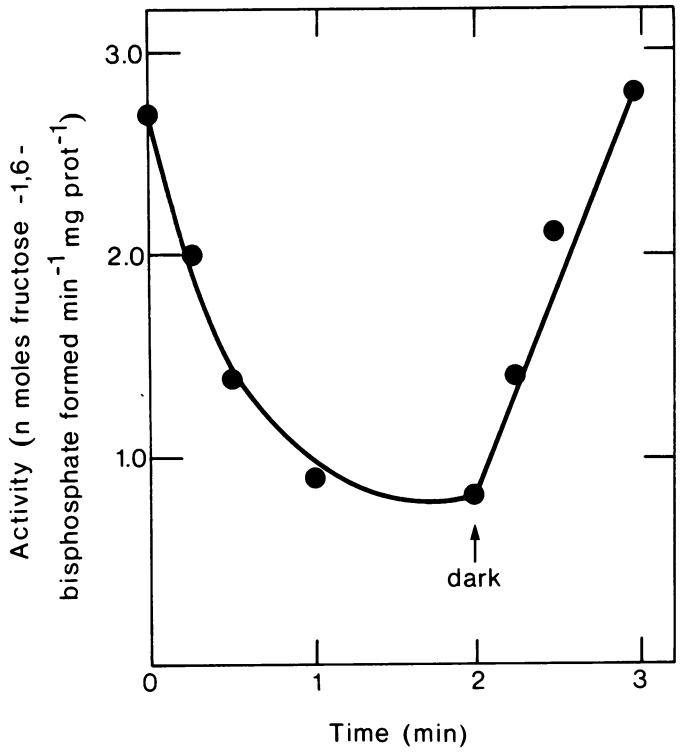

FIG. 1. Light invactivation of P-fructokinase in a broken pea leaf chloroplast preparation. The broken chloroplast preparation was preincubated for 10 min in the dark and then irradiated (5,500 ft-c). At set time intervals, aliquots were removed and diluted 10-fold in ice cold deionized H_2O. After 2 min, the broken chloroplasts were removed from the light. In this experiment, the reaction was initiated by addition of ATP and was run against a reference cuvette which did not contain ATP. Similar results were obtained in other experiments in which fructose-6-P was used to initiate the reaction and the reaction was run against a reference cuvette without fructose-6-P. Chl concentration was 0.251 mg ml^{-1}. The effect of DCMU, arsenite, and sulfite on light modulation was also determined. The broken chloroplast preparation was preincubated for 10 min in the dark in the presence of 2 μM DCMU, 100 μM arsenite, or 10 μM sulfite and then irradiated. These compounds at these concentrations totally inhibited light inactivation of P-fructokinase.

Table I. *Light Modulation of Chloroplast Enzymes Involved in Starch Breakdown and Glucose Metabolism*

Light treatment was for 10 min with 5500 ft-c white light as described under "Materials and Methods." Chl concentrations during modulation in mg/ml were: phosphorylase, 0.14; phosphoglucomutase, 0.72; phosphoglucoisomerase, 0.4. Glucose-6-P dehydrogenase from ref. 5; fructose bisphosphatase data from ref. 4. The P-fructokinase data is from this paper; irradiation was only for 2 min.

Enzyme	Dark Activity	Light Activity	Light Effect	
			Inactivation	Activation
	nmol product min^{-1} mg^{-1} prot		%	
Phosphorylase	0.17	0.11	35	
Phosphoglucomutase	9.9	2.7	73	
Glucose-6-P dehydrogenase	46	34	26	
Phosphoglucoisomerase	12.5	3.5	72	
P-Fructokinase	2.7	0.8	68	
Fructose bisphosphatase	4.4	6.6		150

the LEM system or the thioredoxin system (2). The high sensitivity to sulfite is probably indicative of the involvement of the LEM system in light inactivation of this chloroplastic enzyme.

We concluded that P-fructokinase is controlled by the light-mediated process which regulates both glycolysis and the oxidative pentose phosphate pathway as well as photosynthetic carbon metabolism in the chloroplast.

Usually maximal velocity, but not K_m, is affected by light modulation (1). The same is true for chloroplastic P-fructokinase. The K_m (fructose-6-P) of the light form is 0.126 ± 0.029 mM and of the dark form is 0.119 ± 0.017 mM (weighted means of two separate determinations). This is similar to the value reported by Garland and Dennis (12) for the castor bean endosperm plastid enzyme under similar conditions. The pH dependency of chloroplastic glucose-6-P dehydrogenase (8) and of NADP-linked glyceraldehyde-3-P dehydrogenase is affected by light modulation. No effect on the optima of the chloroplastic P-fructokinase was seen in the present experiments (data not shown). The pH optimum was around 7.7. The pH optima of the castor bean (12) and spinach (17) plastid enzymes are also at 7.7.

Sulfate inhibits plastid (11, 17), but not cytosolic (11) P-fructokinases. In a previous paper from this laboratory (13) whole leaf P-fructokinase was reported to be light inactivated. The $(NH)_2SO_4$ present in the coupling enzyme mixture used in those experiments causes about 60% inhibition of the activity of the chloroplast enzyme (data not shown). Since P-fructokinase was totally light inactivated in most of the experiments reported in that paper, it seems that cytosolic P-fructokinase must be essentially totally light inactivated. A fraction of the total activity measured in those experiments may, however, have been chloroplastic.

The three enzymes of starch catabolism which precede P-fructokinase are also light inactivated and dark activated while fructosebisphosphatase is light activated (Table I). It seems that the flow of carbon from fructose-6-P into starch and into exportable triose-P and pentose phosphate pathway intermediates is restricted in the light. The major effect of the inactivation of these enzymes may then be to dam up the flow of carbon out of the reductive pentose phosphate cycle, thus increasing cycle intermediates during the induction period in photosynthetic CO_2 fixation.

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