Plant Physiol. (1979) 64, 491–494
0032-0889/79/64/0491/04/$00.50/0

Modulation of Chloroplast Fructose-1,6-bisphosphatase Activity by Light[1]

Received for publication January 29, 1979 and in revised form May 10, 1979

LOUISE E. ANDERSON[2], HAE-MIN CHIN[3], AND VIMAL K. GUPTA[4]
Department of Biological Sciences, University of Illinois at Chicago Circle, Chicago, Illinois 60680

ABSTRACT

Inhibitor experiments indicate that light effect mediator$_{II}$ which is reductively activated by transfer of electrons from the photosynthetic electron transport system at or beyond ferredoxin, is involved in activation by light of fructose-1,6-bisphosphatase in the pea plant. Activation proceeds optimally when the pH is low and Mg^{2+} is 10 millimolar. Modulation by light results in increases in maximal velocity, apparently as a result of changes in enzyme conformation. Pea leaf thylakoids are effective in modulating the activity of glyceraldehyde-3-phosphate dehydrogenase but not of fructose-1,6-bisphosphatase or glucose-6-phosphate dehydrogenase in Kalanchoë stromal extracts. There is apparently species specificity for modulation of some, but not all, of the modulatable enzymes.

The reductive pentose phosphate cycle enzyme fructose-1,6-bisphosphatase (EC 3.1.3.11) is activated by light in intact or broken pea (*Pisum sativum*) and spinach (*Spinacia oleracea*) chloroplasts (12–14, 18). In reconstituted chloroplast systems activation requires thylakoid membranes, ferredoxin, thioredoxin, and ferredoxin-thioredoxin reductase (11, 24, 26).

In *P. sativum* modulation by light of chloroplast carbon metabolism enzyme activity is mediated by membrane-bound LEM[5] systems (2). LEM$_I$ accepts electrons from the photosynthetic electron transport system on the reducing side of PSI prior to ferredoxin. It is involved in the activation of glyceraldehyde-3-P dehydrogenase, ribulose-5-P kinase, and NADP-linked malic dehydrogenase, and in the inactivation of glucose-6-P dehydrogenase. LEM$_{II}$ accepts electrons at the level of, or after, ferredoxin, and activates sedoheptulose-1,7-bisphosphatase (2). The present experiments indicate that the LEM$_{II}$ system is also involved in the activation of fructose-1,6-P_2 phosphatase.

We have previously characterized LEM$_{I(G6P\text{-}D)}$ and studied the kinetic properties of glucose-6-P dehydrogenase from light- and dark-treated chloroplasts (3). One purpose of the present experiments was to characterize LEM$_{II(FBPase)}$ and to examine the kinetic properties of fructose-1,6-bisphosphatase from dark- and light-treated chloroplasts.

Four chloroplast enzymes, glyceraldehyde-3-P dehydrogenase, ribulose-5-P kinase, NADP-linked malic dehydrogenase, and sedoheptulose-1,7-bisphosphatase, are activated by light in the CAM plant *Kalanchoë* (16). A further purpose of the present experiments was to determine whether or not the more active (light) and less active (dark) forms of glyceraldehyde-3-P dehydrogenase would be interconvertible by both *Kalanchoë* and *Pisum* LEM systems, and whether the *Kalanchoë* LEM system could activate fructose bisphosphatase in peak leaf stromal extracts.

MATERIALS AND METHODS

Pea (*P. sativum* L., var. Little Marvel) and *Kalanchoë* cv. Tetra Vulcan plants were grown in a greenhouse as in our previous experiments (3, 16).

Preparation of Extracts and Chloroplasts. Pea leaf chloroplasts, and reconstituted chloroplast systems, were prepared as described previously (2) except that Mg^{2+} was omitted from the resuspension media in the experiments where the effect of Mg^{2+} on light activation was studied and, in pH optimum experiments, the Hepes concentration in the resuspension media was 5 mM.

Kalanchoë stromal and thylakoid fractions were prepared by the same method as were pea leaf fractions except that the isotonic chloroplast isolation medium was 2% (w/v) in PEG 6000 and the $MgCl_2$ concentration in the hypotonic buffer solution was 2 mM. In the experiments with the mixed *Kalanchoë Pisum* systems, $MgCl_2$ was 2 mM throughout.

Enzyme Assays. Fructose-1,6-bisphosphatase activity was measured either by the method of Smillie (14, 24) or by the method of Preiss and Greenberg (21) but without albumin in the assay mixture. In the latter case NADPH formation was sometimes followed by increase in fluorescence (340 + 360 nm exciting light) monitored by an Eppendorf photometer 1100 adapted for fluorimetry. Assay pH was 8.3 or 8.8, as noted, and, except in the determination of maximal velocity (Table I), Mg^{2+} concentration was 10 mM. All assays were conducted at room temperature (about 20 C).

Glucose-6-P dehydrogenase, NADP-linked glyceraldehyde-3-P dehydrogenase, and malic dehydrogenase were assayed as described previously (16). The transketolase assay mixture contained 25 μmol Tris-HCl (pH 7.6), 0.1 μmol NADH, 3 μmol $MgCl_2$, 0.5 μmol xylulose-5-P, 0.5 μmol ribose-5-P, and 0.12 nmol thiamine pyrophosphate in a total volume of 1 ml. Triose-P isomerase and α-glycerol-P dehydrogenase were added to couple the formation of glyceraldehyde-3-P to NADH oxidation. Change at A_{340} was followed using a Gilford 2400 recording spectrophotometer.

Activation or Inactivation by Light. LEM activity was assayed

[1] This research was supported by National Science Foundation Grants GB 28160, PCM 75-02281, PCM 75-17068, and PCM 77-08355 and Department of Health, Education and Welfare Biomedical Support Grant RR 07158 01.

[2] Address reprint requests to this author.
[3] Present address: Biology Department, Northwestern University, Evanston, Illinois 60201.
[4] Present address: Botany Department, University of Nairobi, Kenya. Permanent address: Biology Department, G. B. Pant University, Pantnagar, India.

[5] Abbreviations: LEM: light-effect-mediator; LEM$_{I(GGP\text{-}D)}$: LEM system which inactivated glucose-6-P dehydrogenase; LEM$_{I(G3P\text{-}D)}$: LEM system which activates NADP-linked glyceraldehyde-3-P dehydrogenase; LEM$_{II(FBPase)}$: LEM system which activates fructose-1,6-P_2 phosphatase; CCCP: carboxyl cyanide *m*-chlorophenylhydrazone; DSPD: disalicylidenepropanediamine; Diquat: 1,1′-ethylene-2,2′-dipyridylium dibromide; DTNB: 5,5′-dithiobis(2-nitrobenzoic acid).

by the method of Anderson and Avron (2), with some modification. The light source was a General Electric 300-w, 120-v cool beam flood lamp (No. 300 Par 56/WFL), 35 cm distant. Light intensity was 6,000 ft-c. The reconstituted broken chloroplast systems were preincubated in the dark for 20 min on ice and then for 5 min at 25 C. Initial velocity of light modulation (activation or inactivation of the indicated enzyme), denoted $v_{0(LEM)}$, was extrapolated from change in enzyme activity after 0, 1, 2, 3, and 4 min illumination and is expressed in nmol product formed min^{-2} mg stromal $protein^{-1}$ (*i.e.* change in enzyme activity per unit time).

Determination of pH Optima and Kinetic Constants. Procedures used for pH determinations and estimation of maximal velocity were as described previously (3).

Protein and Chl Estimation. Protein and Chl concentrations were estimated by the methods used earlier (2).

Chemicals. Biochemicals and CCCP were obtained from Sigma Chemical Company. DCMU was the product of Pfaltz and Bauer, Inc. and was recrystalized from ethanol prior to use. DSPD was the product of Fluka; Diquat, of Plant Production Ltd., Jeolots's Hills Research Station, Yalding, England. PEG 6000 was obtained from General Biochemicals and was used without further purification. Other chemicals were the highest grade commercially available. Pea seeds were obtained from Northrup and King, Chicago. *Kalanchoë* plants were purchased from George Ball Company, Chicago.

RESULTS AND DISCUSSION

Activation of fructose-1,6-bisphosphatase by light in intact chloroplasts results in an increase in maximal velocity of the enzyme (Table I). Light modulation, likewise, affects the V_{max} of the chloroplastic forms of glyceraldehyde-3-P dehydrogenase and glucose-6-P dehydrogenase (3, 19). Treatment of stromal extracts with DTT results in similar changes (Table I; ref. 3). Low activity levels in Table I reflect low pH and low Mg^{2+} concentrations used in the assays. Conditions in the first experiment were chosen to approximate *in vivo* conditions in the illuminated leaf.

Reports from other laboratories indicate that in spinach activation of fructose-1,6-bisphosphatase by light is not observed when the Mg^{2+} concentration in the enzyme assay is above 4 mM (11) and that activation by DTT cannot be observed when assay pH is 8.8 (10, 15). The data in Table I indicate that in pea leaf chloroplasts fructose-1,6-bisphosphatase activation is measurable when Mg^{2+} concentration in the enzyme assay is 5 mM or when the assay pH is 8.8. We have also observed activation using 10 mM $MgCl_2$ and pH 8.8 buffer in the enzyme assay (see Table II and elsewhere).

Table I. *Maximal Velocity for Fructose-1,6-bisphosphatase from Light- and Dark-treated Pea Leaf Chloroplasts and from DTT-treated Stromal Extracts*

In experiment I chloroplasts suspended in 0.33 M sorbitol, 50 mM Hepes (K^+), 2 mM $MgCl_2$, 1 mM EDTA (pH 7.2) were irradiated for 10 min at 25 C; the spectrophotometric assay of Preiss and Greenberg (21) was used, with Mg^{2+}, 5 mM and pH 8.0. In experiments II and III chloroplasts were irradiated for 2 min on ice with 725 ft-c white light. DTT treatment consisted of making extracts of dark-treated chloroplasts 50 mM in DTT and then allowing the extract to remain on ice for at least 30 min. In these two experiments enzyme assay Mg^{2+} concentration was 0.25 mM and pH was 8.8. The two-step colorimetric assay of Smillie (25) was used. The fructose-1,6-P_2 concentrations used in all three experiments were 0.4, 2.32, 4.24, 6.16, 8.08, and 10 mM. Low activity levels reflect low pH and low Mg^{2+} concentrations used in these experiments. Conditions in experiment I presumably approximate *in vivo* conditions in the illuminated leaf.

Experiment	Dark	Light	DTT
	nmol product formed/mg protein·min		
I	2.9 ± 0.4	4.7 ± 0.7	
II	1.9 ± 0.2	3.3 ± 0.2	
III	2.8 ± 0.7	4.8 ± 1.1	7 ± 1

Table II. *Levels of Reductive Pentose-P Cycle Enzymes in Pea Leaf Chloroplasts*

Results are mean values for triplicate activity determinations. Similar results were obtained in duplicate experiments. Values are not available for ribulose-1,5-P_2 carboxylase, ribose-5-P isomerase, xylulose-5-P 3-epimerase, or triose-P isomerase.

Enzyme	Dark Activity	Light Activity	Activation	Source
	nmol product formed/mg stromal protein·min		*X-fold*	
P-glycerate kinase	570			20
NADP-linked glyceraldehyde-3-P dehydrogenase	100	240	2.4	3
Aldolase (with fructose-1,6-P_2 as substrate)	83			4
Fructose-1,6-bisphosphatase[a]	4.4	6.7	1.5	Table V
Transketolase	140			
Aldolase (with sedoheptulose-1,7-P_2 as substrate)[b]	39			4,7
Sedoheptulose-1,7-bisphosphatase	2.5	4.5	1.8	1
Ribulose-5-P kinase	82	130	1.6	Table V

[a] Assayed at pH 8.2. At pH 8.8 and 10 mM $MgCl_2$ and after light activation, we have observed activities as high as 70 nmol fructose-6-P produced/mg protein·min.

[b] Calculated from the activity ratios for the two substrates with the purified enzyme (7) and the levels of fructose-1,6-P_2 aldolase activity in crude stromal extracts (4).

Table III. *Effect of DSPD on Light Activation of Fructose-1,6-bisphosphatase in Intact and Broken Pea Leaf Chloroplasts*

Chloroplasts were incubated in 0.33 M sorbitol, 2 mM $MgCl_2$, 2 mM EDTA, 50 mM Hepes (K^+) (pH 7.2) (intact chloroplasts), or in 50 mM Hepes (K^+), 1 mM EDTA, 10 mM KCl, 10 mM $MgCl_2$ (pH 7.3) (broken chloroplasts) in the presence or absence of 0.8 mM DSPD. After 5-min preincubation samples were light- or dark-treated for an additional 10 min at 25 C. Fructose-1,6-bisphosphatase activity was assayed using the spectrophotometric assay of Preiss and Greenberg (21). Assay pH was 8.8 and $MgCl_2$ concentration was 10 mM.

Experiment	Chloroplasts	Dark Activity	Activation		DSPD Inhibition of Activation
			Control	DSPD	
		nmol/min·mg stromal protein	*X-fold*		%
I	Intact	14.2	1.33	1.12	64
II	Broken	19.0	1.41	1.08	80

Fructose-1,6-bisphosphatase and sedoheptulose-1,7-bisphosphatase may be the rate-limiting reductive pentose-P cycle enzymes in the pea leaf chloroplast (Table II). The 1.5-fold increase in the maximal velocity of the hexose bisphosphatase would result in a similar increase in reductive pentose-P cycle activity at saturating substrate concentrations *in vivo*.

Modulation by light is thought to involve thylakoid membrane-bound LEM systems. The LEMs are reductively activated by transfer of electrons from the electron transport system (2). LEM_I is DCMU- and Diquat-sensitive, but DSPD-insensitive, and hence must be located on the reducing side of PSI before ferredoxin. LEM_{II} is sensitive to DSPD as well as to DCMU and Diquat and therefore must be located at or beyond ferredoxin. LEM_{II} is involved in sedoheptulose bisphosphatase activitation (2) and has been thought to be involved in fructose bisphosphatase activation because experiments from Buchanan's laboratory (11, 22) indicate that ferredoxin is required for light activation of both phosphatases. The experiments in Table III indicate that DSPD does affect the LEM system for activation of fructose-1,6-bisphosphatase.

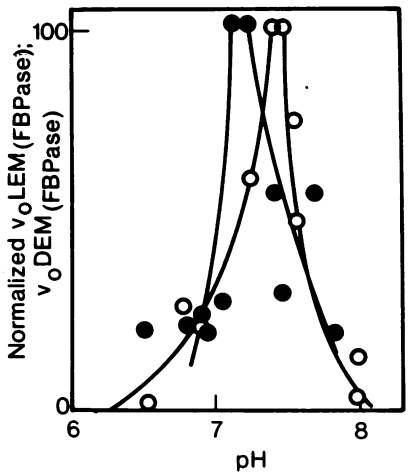

FIG. 1. pH dependence of $LEM_{II(FBPase)}$ (○) and of dark inactivation system, $DEM_{(FBPase)}$ (●). Data shown are a composite of data from three light activation experiments and four dark inactivation experiments. Initial velocity of modulation in each experiment was plotted *versus* pH. Values were then normalized on a scale of 0 to 100 and replotted. $MgCl_2$ concentration was 10 mM in all experiments. Particulate fraction was added to a final concentration of 10 μg Chl/ml. Stromal protein concentrations in activation assays were 0.71, 0.7, and 0.86 mg/ml; in dark inactivation assays, 0.82, 1.2, 1.3, and 2.4 mg/ml. Light exposure prior to dark inactivation was for 2 min at pH 7.4 in 5 mM potassium Hepes, 10 mM $MgCl_2$. Fructose-1,6-bisphosphatase activity was monitored using the coupled enzyme assay of Preiss and Greenberg (21). Light and dark modulation of fructose-1,6-bisphosphatase activity is markedly pH-dependent.

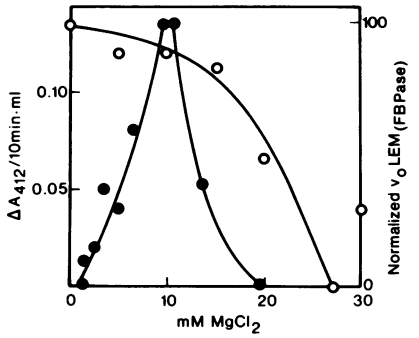

FIG. 2. Mg^{2+} dependence of $LEM_{II(FBPase)}$ (●) and of light-dependent DTNB reduction (○). The system for activation of LEM_I is similar to, and possibly identical with, the system for light-dependent reduction of DTNB. $LEM_{II(FBPase)}$ data are a composite of two experiments. Initial velocity of modulation relative to maximal velocity of modulation in each experiment was plotted *versus* Mg^{2+} concentration. Values were normalized and replotted to give this curve. Particulate fraction was adjusted to give a final concentration of 10 μg Chl/ml. Activation assay pH was 7.4. Stromal protein concentration in activation assays was 0.65 and 1.4 mg/ml. Fructose-1,6-bisphosphatase activity was monitored using the two-step colorimetric assay of Smillie (25) with modifications used previously for assay of sedoheptulose-1,7-bisphosphatase. For details of DTNB photoreduction experiments see reference 6. Each cuvette contained 25 μmol potassium Hepes, 1 μmol EDTA, 1 μmol DTNB (pH 7.4), chloroplast particulate fraction (40 μg Chl), and $MgCl_2$ at the indicated concentrations in a total volume of 1 ml. This experiment was repeated three times with consistent results. Apparently the LEM_{II} system is both Mg^{2+}-dependent and Mg^{2+}-inhibited, while the system for DTNB reduction is sensitive only to very high Mg^{2+} concentrations.

Hence the hexose bisphosphatase, like the heptulose bisphosphatase, is LEM_{II}-activated.

The pH optimum for LEM_{II}-catalyzed activation of fructose-1,6-bisphosphatase is 7.4 (Fig. 1). This pH dependence curve is essentially identical with those for light activation of ribulose-5-P kinase (16) and for light inactivation of glucose-6-P dehydrogenase mediated by the LEM_I system (3), and for the light-dependent reduction of the disulfide bond of DTNB (6). In a chloroplast exposed to intense illumination the pH of the stroma is around 8 (17). In a weakly illuminated chloroplast as, for example, at dawn, the pH of the stroma is lower (17). In the dark stromal pH is around 7.1 (17). The LEM_{II} system, like the LEM_I system, must be maximally active at dawn and when light intensity is very low.

Low concentrations of Mg^{2+} are required for LEM_{II}-mediated activation of fructose-1,6-bisphosphatase. At high concentrations Mg^{2+} inhibits (Fig. 2). In contrast Mg^{2+} does not appear to be required for LEM_I-mediated inactivation of glucose-6-P dehydrogenase (JX Duggan, LE Anderson, unpublished data). DTNB, like LEM_I, accepts electrons from the electron transport system on the reducing side of PSI before ferredoxin (6): Mg^{2+} is not

Table IV. *Light Modulation in Mixed Kalanchoë-Pisum Thylakoid-Stromal Systems*

Stromal and thylakoid fractions were prepared from *Kalanchoë* and pea leaf chloroplasts and combined as indicated. Chl concentration was about 48 μg/ml (constant within each experiment). The pea leaf stromal protein concentration in the reconstituted systems was 5 mg/ml in the experiment with glyceraldehyde-3-P dehydrogenase and 5.5 mg/ml in the other experiments. The *Kalanchoë* stromal protein concentration was 3.1 mg/ml in the experiment with glyceraldehyde-3-P dehydrogenase and 2.2 in the other two experiments. Fructose-1,6-bisphosphatase assay pH was 8.8. Activity of the modulatable enzyme ($v_{o(enz)}$) before light treatment is expressed as nmol substrate consumed mg stromal protein·min. Activity of the LEM system ($v_{o(LEM)}$) is expressed as change in activity of the modulated enzyme per minute, which is nmol enzyme substrate consumed mg stromal $prot^{-1}$ min^{-2}.

System		Enzyme					
		NADP-linked glyceraldehyde-3-P dehydrogenase		Glucose-6-P dehydrogenase		Fructose-1,6-bisphosphatase	
Stroma	Thylakoid	Vo(enz)	Vo(LEM)	Vo(enz)	Vo(LEM)	Vo(enz)	Vo(LEM)
Pea	Pea	30	75	10	2.7	18	87
Pea	*Kalanchoë*	44	52	12	0	20	0
Kalanchoë	*Kalanchoë*	70	83	10	0	80	0
Kalanchoë	Pea	72	75	10	0	80	0

Table V. *Effect of Trypsin Digestion on Light-modulated Chloroplast Enzymes*

Enzymes were light-activated or inactivated in intact chloroplasts as previously described (2). Light intensity was 5,500 ft-c; exposure time was 5 min; temperature was maintained at 25 C. Chloroplasts were lysed osmotically (2) and membrane particles were removed by centrifugation for 10 min at 20,000*g*. Ten μg trypsin was added per ml supernatant solution. Aliquots were removed at 0-, 10-, and 20-min intervals and soybean trypsin inhibitor (50 μg/ml) was added to stop the digestion. NADP-linked malic dehydrogenase dark activity was 1.3 nmol/min·mg stromal proteins; activation was 5.6-fold. Ribulose-5-P kinase dark activity was 82 nmol/min·mg stromal protein; activation was 1.6-fold. Fructose-1,6-bisphosphatase dark activity was 4.4 nmol/min·mg stromal protein; activation was 1.5-fold. Glucose-6-P dehydrogenase dark activity was 1.8 nmol/min·mg stromal protein; inactivation was 80%.

Chloroplast Treatment	Digestion Time	Stromal Enzyme			
		NADP-linked malic dehydrogenase	Ribulose-5-P kinase	Fructose-1,6-bisphosphatase	Glucose-6-P dehydrogenase
		Protease inactivation			
	min	%			
Dark	10	3	0	0	51
	20	7	0	21	67
Light	10	21	12	34	39
	20	37	19	63	50

required for light-dependent DTNB reduction and at high levels Mg^{2+} inhibits DTNB reduction (Fig. 2). Apparently only the LEM_{II} system required Mg^{2+} for activity.

Several carbon metabolism enzymes are activated by light in the CAM plant *Kalanchoë* (14). When the thylakoid membranes from this plant are treated with arsenite they lose capacity for activating NADP-linked glyceraldehyde-3-P dehydrogenase and malic dehydrogenase; likewise, sulfite inactivates the *Kalanchoë* thylakoid-membrane-bound activation system (data not shown). The results of experiments with the inhibitors DCMU, Diquat, CCCP and DSPD (data not shown) are essentially the same as those obtained for the pea leaf LEM_I system (2). Clearly the pea leaf and *Kalanchoë* LEM_I systems are very similar.

It seemed possible that the pea leaf and *Kalanchoë* LEM systems would be interchangeable. The stromal NADP-linked glyceraldehyde-3-P dehydrogenases of both plants are indeed activated by both LEM systems (Table IV). Apparently these two $LEM_{I(G3P\text{-}D)S}$ have little specificity or the modulatable glyceraldehyde-3-P dehydrogenases in the two species are very similar.

In contrast the *Kalanchoë* LEM system does not activate pea leaf stromal fructose-1,6-bisphosphatase nor does it inactivate pea leaf chloroplast glucose-6-P dehydrogenase (Table IV). Neither enzyme in *Kalanchoë* stromal extracts is affected by the pea leaf LEM system just as neither is modulated by light in the intact *Kalanchoë* chloroplast (16). Since the two pea leaf enzymes are not affected by the *Kalanchoë* LEM system, this system does differ from the pea leaf LEM system. Since *Kalanchoë* glucose-6-P dehydrogenase and fructose-1,6-bisphosphatase are not affected by the pea leaf LEM system and are not DTT-sensitive (16), it would appear that there are significant differences, either in primary structure or in conformation, in these two enzymes in pea and *Kalanchoë*. Ferredoxin, thioredoxin reductase, and thioredoxins with partial specificity for activation of specific enzymes have been implicated in modulation by light (12, 24, 26). Recent work in this laboratory confirms the requirement for a stromal factor which may be identical with the reductase, but not the thioredoxin requirement (8, 9). Lack of glucose-6-P dehydrogenase- and fructose-1,6-bisphosphatase-specific soluble factors could account for the results obtained in the pea leaf thylakoid-*Kalanchoë* stroma system, but not for the results obtained in the *Kalanchoë* thylakoid-pea leaf stroma system. Clearly *Kalanchoë* LEM_{II} is not capable of activating fructose-1,6-bisphosphatase in the pea leaf stromal system, and *Kalanchoë* LEM_I cannot inactivate glucose-6-P dehydrogenase in the pea leaf stromal system.

If modulation by light involved a change in the conformation of the modulated enzymes, then there might also be a difference in the susceptibility of the light and dark forms of the enzyme to proteolytic digestion. When we subjected stromal extracts from light- or dark-treated chloroplasts to trypsin digestion, the light-activated forms of NADP-linked malic dehydrogenase, ribulose-5-P kinase, and fructose-1,6-bisphosphatase were inactivated by the protease (Table V). Both forms of glucose-6-P dehydrogenase were sensitive to proteolytic digestion, with the dark form being slightly more sensitive. These data suggest that light modulation involves a change in conformation of the modulated enzymes. Schürmann and Wolosiuk (23) made similar observations in a study of the thioredoxin-activated form of fructose-1,6-bisphosphatase. Although NADP-linked glyceraldehyde-3-P dehydrogenase was not affected by trypsin in these experiments (data not shown), earlier experiments with this enzyme suggest that it, too, undergoes a change in conformation during activation (5). It remains to be seen whether this conformational change is accomplished by reduction of disulfide bonds (26) or by thiol, disulfide exchange (6).

Acknowledgments—The maximal velocity estimations (Table I) were made by J. X. Duggan and M. J. Hansen. S. C. Nehrlich determined the effect of Mg^{2+} on the light-dependent reduction of DTNB (Fig. 2). J. McCorkle, L. Sykora, and staff at the University of Illinois, Chicago Circle Greenhouse, grew the pea plants. The computer facilities at the University of Illinois, Chicago Circle Computer Center were used in analyzing the data in Table I. We thank M. Avron for the Diquat, M. Gibbs for the DSPD, and M. Gassman for the DCMU used in these experiments.

LITERATURE CITED

1. ANDERSON LE 1974 Activation of pea leaf chloroplast sedoheptulose 1,7-diphosphate phosphatase by light and dithiothreitol. Biochem Biophys Res Commun 59: 907–913
2. ANDERSON LE, M AVRON 1976 Light modulation of enzyme activity in chloroplasts. Generation of membrane-bound vicinal-dithiol groups by photosynthetic electron transport. Plant Physiol 57: 209–213
3. ANDERSON LE, JX DUGGAN 1976 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 enzymes. Plant Physiol 58: 135–139
4. ANDERSON LE, RL HEINRIKSON, C NOYES 1975 Chloroplast and cytoplasmic enzymes. VII. Subunit structure of pea leaf aldolases. Arch Biochem Biophys 169: 262–268
5. ANDERSON LE, T-C LIM 1972 Chloroplast glyceraldehyde 3-phosphate dehydrogenase: light-dependent change in the enzyme. FEBS Lett 27: 189–191
6. ANDERSON LE, SC NEHRLICH, M-L CHAMPIGNY 1978 Light modulation of enzyme activity. Activation of the light effect mediators by reduction and modulation of enzyme activity by thiol-disulfide exchange. Plant Physiol 61: 601–605.
7. ANDERSON LE, I PACOLD 1972 Chloroplast and cytoplasmic enzymes. IV. Pea leaf fructose-1,6-diphosphate aldolases. Plant Physiol 49: 393–397
8. ASHTON AR, LE ANDERSON 1979 Partial resolution of the light effect mediator system of pea leaf chloroplasts. Abstracts, XI International Congress of Biochemistry. In press
9. ASHTON AR, LE ANDERSON 1979 Reconstitution of the light activation system for pea leaf chloroplast malate dehydrogenase. Plant Physiol 64:S. S-25
10. BAIER D, E LATZKO 1975 Properties and regulation of C-1-fructose-1,6-diphosphatase from spinach chloroplasts. Biochim Biophys Acta 396: 141–148
11. BUCHANAN BB, P SCHÜRMANN, PP KALBERER 1971 Ferredoxin-activated fructose dephosphatase of spinach chloroplasts. Resolution of the system, properties of the alkaline fructose diphosphatase component, and physiological significance of the ferredoxin-linked activation. J Biol Chem 246: 5952–5959
12. BUCHANAN BB, RA WOLOSIUK, NA CRAWFORD, BC YEE 1978 Evidence for three thioredoxins in leaves. Plant Physiol 61: S-38
13. CHAMPIGNY M-L, E BISMUTH 1976 Role of photosynthetic electron transfer in light activation of Calvin cycle enzymes. Physiol Plant 36: 95–100
14. DUGGAN JX, LE ANDERSON 1975 Light-regulation of enzyme activity in *Anacystis nidulans*. Planta 122: 293–297
15. EL-BALDRY AM 1974 Hexosediphosphatase from spinach chloroplasts. Purification, crystallization and some properties. Biochim Biophys Acta 333: 366–377
16. GUPTA VK, LE ANDERSON 1978 Light modulation of the activity of carbon metabolism enzymes in the Crassulacean acid metabolism plant *Kalanchoë*. Plant Physiol 61: 469–471
17. KELDT HW, K WERDAN, M MILOVANCEV, G GELLER 1973 Alkalization of the chloroplast stroma caused by light-dependent proton flux into the thylakoid space. Biochim Biophys Acta 314: 224–241
18. KELLY GJ, G ZIMMERMAN, E LATZKO 1976 Light-induced activation of fructose-1,6-bisphosphatase in isolated intact chloroplasts. Biochem Biophys Res Commun 70: 193–201
19. MELANDRI BA, P PUPILLO, A BACCARINI-MELANDRI 1970 D-Glyceraldehyde-3-phosphate dehydrogenase in photosynthetic cells. I. The reversible light-induced activation *in vivo* of NADP-dependent enzyme and its relationship to NAD-dependent activities. Biochim Biophys Acta 220: 178–189
20. PACOLD I, LE ANDERSON 1975 Chloroplast and cytoplasmic enzymes. VI. Pea leaf 3-phosphoglycerate kinases. Plant Physiol 55: 168–171
21. PREISS J, E GREENBERG 1971 Spinach leaf D-fructose 1,6-diphosphate 1-phosphohydrolase (FDPase) EC 3.1.3.11. Methods Enzymol 23: 691–696
22. SCHÜRMANN P, BB BUCHANAN 1975 Role of ferredoxin in the activation of sedoheptulose diphosphatase in isolated chloroplasts. Biochim Biophys Acta 376: 189–192
23. SCHÜRMANN P, RA WOLOSIUK 1978 Studies on the regulatory properties of chloroplast fructose-1,6-bisphosphatase. Biochim Biophys Acta 522: 130–138
24. SCHÜRMANN P, RA WOLOSIUK, VD BREAZEALE, BB BUCHANAN 1976 Two proteins function in the regulation of photosynthetic CO_2 assimilation in chloroplasts. Nature 263: 257–258
25. SMILLIE RM 1964 Plant fructose-1,6-diphosphatase. *In* RW McGilvery, BM Pogell, eds, Fructose-1,6-diphosphatase and its role in gluconeogenesis. Port City Press, Baltimore, pp 31–41
26. WOLOSIUK RA, BB BUCHANAN 1977 Thioredoxin and glutathione regulate photosynthesis in chloroplasts. Nature 266: 565–567