ACTIVATION OF PEA LEAF CHLOROPLAST SEDOHEPTULOSE 1,7-DI-PHOSPHATE PHOSPHATASE BY LIGHT AND DITHIOTHREITOL

Louise E. Anderson

Department of Biological Sciences
University of Illinois at Chicago Circle
Chicago, Illinois 60680

Received June 18,1974

Light activates a Mg++ dependent enzyme in pea (Pisum sativum) seedlings which liberates phosphate from sedoheptulose 1,7-diphosphate. This enzyme, which is located exclusively in the chloroplast, appears to be the sedoheptulose diphosphatase involved in the reductive pentose phosphate cycle in green plants.

Light-mediated changes in the steady state levels of sedoheptulose-1,7-diP and sedoheptulose-7-P suggest that the activity of sedoheptulose-1,7-diP phosphatase is light modulated in green plants (1, 2). Although several enzymes of photosynthetic carbon metabolism have been shown to be activated by light in vivo and by dithiothreitol in vitro (3-12), and two enzymes of catabolic carbon metabolism have been shown to be inactivated by light and by dithiothreitol (13-16), activation of sedoheptulose-1,7-diphosphate phosphatase has not been reported previously. Initial attempts to activate the enzyme described by Racker and Schroeder (17) in extracts of pea (Pisum sativum L.) leaves were futile. I now wish to report the activation of a chloroplast specific sedoheptulose-1,7-diP phosphatase in vivo by light and in vitro by dithiothreitol. This enzyme, which appears to be distinct from the enzyme described by Racker and Schroeder, requires Mg++ for activity and has an alkaline pH optimum.

MATERIALS AND METHODS

Pea (Pisum sativum L., var. Little Marvel) plants were grown 9 to 12 days in a green house under natural light in a vermiculite, soil mixture or in absolute darkness for 7 to 10 days in vermiculite. Dark-treated plants were placed in the dark overnight. Light-treated plants were exposed to light (4×10^4 ergs/cm^2.sec) from two GE 30 W, 115 volt reflector flood lamps 18 cm distant for 15 minutes. Extracts of whole leaves and of etiolated shoot tips were prepared by grinding tissue in 100 mM pH 8.1 Tris, HCl with a glass tissue grinder, in darkness for dark-grown and dark-treated plants. Chloroplasts, and cytoplasmic fractions, were prepared as described previously (18). Chloroplasts were lysed by suspension in 1% Triton X-100 in 100 mM Tris, HCl buffer. For dithiothreitol treatment extracts were made 50 mM in dithiothreitol (with solid dithiothreitol) and were then allowed to stand on ice for at least one hour before activity determination.

Sedoheptulose-diphosphatase activity was assayed by measuring phosphate liberated from sedoheptulose-1,7-diP. Unless otherwise noted the assay mixture contained 0.3 μmoles sedoheptulose-1,7-diP, 50 μmoles Tris, HCl, pH 8.1, 4.5 μmoles $MgCl_2$ and extract in a total volume of 0.25 ml. Reaction was initiated, after 5 minutes preincubation, by the addition of extract, and stopped, after 30 minutes at 25 C, by the addition of 0.4 ml of the phosphate reagent of Taussky et al. (19). After the addition of 0.35 ml H_2O, precipitated protein was removed by centrifugation (5 min., 750 g) and A_{660} was measured using a Gilford 2400 spectrophotometer Fructose-1,6-diP phosphatase activity was measured in the same way except that only 0.25 μmoles fructose-1,6-diP were present in the reaction mixture. Controls, without exogenous substrate, were

Table I. Activation of chloroplastic sedoheptulose-1,7-diP phosphatase by dithiothreitol. Assay tube contained 0.3 μmoles sedoheptulose-1,7-diP or 0.25 μmoles fructose-1,6-diP, 4.5 μmoles $MgCl_2$, 50 μmoles Tris, HCl, pH 8.1, and extract in a total volume of 0.25 ml. Dithiothreitol-treated extracts were made 50 mM with respect to dithiothreitol and allowed to stand on ice for at least one hour prior to activity determination.

	Enzyme Activity, (nmoles Pi produced mg protein^{-1}min^{-1})		Stimulation (activity with dithiothreitol/ activity without dithiothreitol)
	Without dithiothreitol treatment	With dithiothreitol treatment	
Experiment I Sedoheptulose-1,7-diP	3.94	33.8	8.6
Experiment II Sedoheptulose-1,7-diP	1.15	31.9	28
Fructose-1,6-diP	0.23	6.65	29

run in all experiments; activity values are corrected for endogenously liberated phosphate.

Protein concentrations were estimated by the biuret method as previously described (20).

Biochemicals were obtained from Sigma.

RESULTS AND DISCUSSION

Since light-modulated enzymes are often affected by dithiothreitol treatment I first attempted to activate the neutral sedoheptulose-1,7-diP phosphatase described by Racker and Schroeder (17) by incubating whole leaf extracts in 50 mM dithiothreitol. No activation was observed. With chloroplast extracts however a peak of phosphatase activity was found at pH 8.1 and at this pH activation was found with dithiothreitol (Table I). The activated enzyme is restricted to the chloroplast (Table II).

Table II. Distribution of dithiothreitol-activated sedoheptulose-1,7-diP phosphatase in pea leaf chloroplastic and cytoplasmic fractions. Conditions as in Table I.

Fraction	Enzyme Activity (nmoles Pi produced mg protein^{-1}min^{-1})	
	Experiment I	Experiment II
Chloroplast	33.8	31.9
Cytoplasmic	0	4.1

Under the conditions of the assay sedoheptulose-diphosphatase activity is 5-fold higher than fructose-diphosphatase activity, which eliminates the possibility that the activity observed is the result of the action of the hexose-diphosphatase on fructose-1,6-diP present in the sedoheptulose-1,7-diP preparation (about 1% according to the manufacturer) or due to a non-specific phosphatase. Activity with sedoheptulose-7-P is about 7-1/2% of the activity observed with sedoheptulose-1,7-diP; the enzyme would appear to be cleaving the phosphate linked to carbon-1 from the heptulose-diphosphate.

Light-treatment of intact seedlings resulted in about a 2-fold increase in measured enzyme activity (Table III). This confirms the suggestion of Bassham and Kirk (1) that this enzyme is light activated. These workers felt that light regulation might be accomplished through shifts in ion concentration and pH. The enzyme in whole leaf (data not shown) or chloroplast extracts (Table II) is activated by treatment with the strong reducing agent dithiothreitol. Since both light and dithiothreitol activate the enzyme it now seems more likely that light-activation of this enzyme involves a light-mediated reduction of a disulfide bond of the protein.

Table III. Light activation of sedoheptulose-1,7-diP phosphatase in intact pea seedlings. Dark treated plants were placed in the dark overnight. Light-treated plants were exposed to light (4 x 10^4 ergs/cm^2·sec) for 15 minutes. Results are mean values from triplicate assays from three different experiments.

Treatment	Enzyme Activity (nmoles Pi produced mg protein^{-1} min^{-1})		
	Experiment I	Experiment II	Experiment III
Dark	0.53	0.82	0.70
Light	1.13	1.29	1.20
Stimulation	2.1	1.6	1.7

The effect of Mg++ concentrations on the activity of the dithiothreitol-activated phosphatase in chloroplast extracts is shown in Fig. 1. The phosphatase described by Racker and Schroeder did not require Mg++ ions for activity. Mg++ does not appear to be required in the absence of dithiothreitol; it should be noted however that $MgCl_2$ (5mM) is present in the isotonic buffer used for isolation of the chloroplasts and some Mg++ will be present in the chloroplast extract.

In extracts of etiolated pea seedlings four enzymes which are light-activated in chloroplast-containing green plants, ribulose-5-P kinase, NADP-linked and NAD-linked glyceraldehyde-3-P dehydrogenases, and NADP-linked malic dehydrogenase, have been shown to be activated by dithiothreitol (21). Dithiothreitol treatment did not activate the sedoheptulose-diphosphatase present in dark-grown pea seedlings (data not shown). Likewise alkaline fructose-1,6-diP phosphatase was not dithiothreitol activated (see ref. 21). Either the mechanism of activation of the phosphatases is different from the mechanism of activation of

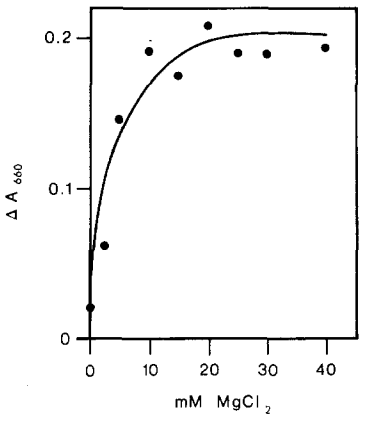

Fig. 1. Effect of Mg++ ion concentration on the activity of dithiothreitol-activated sedoheptulose-diP phosphatase activity in crude chloroplast extracts. Crude chloroplast extract, treated with dithiothreitol and containing 56 μg protein, was incubated with 0.3 μmole sedoheptulose-1,7-diP, 50 μmoles Tris (HCL), pH 8.1, and $MgCl_2$ in a total volume of 0.25 ml for 30 minutes at 25 C.

the other enzymes mentioned above, or the inactive phosphatases are not present in etiolated seedlings.

The sedoheptulose-1,7-diP phosphatase described here, which is activated by light and by dithiothreitol, has an alkaline pH optimum, and is located exclusively in the chloroplast, would appear to be the sedoheptulose-diP phosphatase involved in the reductive pentose phosphate cycle in green plants. The isolation and characterization of the purified enzyme is in progress.

Acknowledgments. I thank James McCorkle and staff for growing the pea plants used in these experiments and Hae-Minh Chin and Kristine Slawinski for assistance with some of the experiments. This work was supported by a grant, GB 28160-A1, from the National Science Foundation.

REFERENCES

1. J. A. Bassham and M. Kirk, in K. Shibata, A. Takamiya, A. T. Jagendorf and R. C. Fuller (Eds.), Comparative Biochemistry and Biophysics of Photosynthesis, University Park Press, State College, Pa., 1968, p. 365.
2. B. Schacter and J. A. Bassham, Plant Physiol. 49 (1972) 411.
3. G. F. Wildner and R. S. Criddle, Biochem. Biophys. Res. Commun. 37 (1969) 952.

4. B. B. Buchanan, P. Schürman and P. P. Kalberer, J. Biol. Chem. 246 (1971) 5952.
5. E. Latzko, R. v. Garnier and M. Gibbs, Biochem. Biophys. Res. Commun. 39 (1970) 1140.
6. E. Steiger, I. Ziegler and H. Ziegler, Planta 96 (1971) 109.
7. H. Ziegler and I. Ziegler, Planta 65 (1965) 369.
8. B. A. Melandri, R. Pupillo and A. Baccarini-Melandri, Biochim. Biophys. Acta 220 (1970) 178.
9. M. D. Hatch and C. R. Slack, Biochem. J. 112 (1969) 549.
10. H. S. Johnson and M. D. Hatch, Biochem. J. 119 (1970) 273.
11. L. E. Anderson and T. C. Lim, FEBS Letters, 27 (1972) 189.
12. M. Avron and M. Gibbs, Plant Physiol. 53 (1974) 136.
13. K. Lendzian and H. Ziegler, Planta 94 (1970) 27.
14. H. S. Johnson, Planta 106 (1972) 273.
15. L. E. Anderson, T. C. L. Ng and K. E. Y. Park, Plant Physiol. in press (1974).
16. R. B. Kachru and L. E. Anderson, submitted.
17. E. Racker and E. A. R. Schroeder, J. Biol. Chem. 74 (1958) 326.
18. R. B. Kachru and L. E. Anderson, Planta, in press (1974).
19. H. Taussky, H. E. Shorr and G. Kurzmann, J. Biol. Chem. 202 (1953) 675.
20. L. E. Anderson and V. R. Advani, Plant Physiol. 45 (1970) 583.
21. L. E. Anderson, Plant Science Lett. 1 (1973) 331.