CHLOROPLAST GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE: LIGHT-DEPENDENT CHANGE IN THE ENZYME

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Received 19 September 1972

1. Introduction

Light, by an unknown mechanism(s), activates four enzymes of the reductive pentose phosphate pathway [1–6] and two enzymes of the C-4 dicarboxylic acid photosynthetic pathway [7, 8], and inactivates the oxidative pentose phosphate pathway enzyme, glucose-6-P dehydrogenase [9]. In some cases treatment with the strong reducing agent dithiothreitol simulates *in vitro* the *in vivo* light effect.

We have now been able to convert one electrophoretically distinct form of TPN-linked glyceraldehyde-3-P dehydrogenase to a second form by irradiation of intact pea (*Pisum sativum*) plants with white light or by treatment of extracts with dithiothreitol. These experiments indicate that light activation involves reduction of the enzyme. The difference in electrophoretic mobility apparently results from differences in charge, and hence conformation, rather than from differences in aggregation of the enzyme.

2. Materials and methods

In these experiments pea (*Pisum sativum*, var. Little Marvel) plants, which had been grown 9 to 12 days in a green house under natural light in a vermiculite, soil mixture, were either placed in the dark overnight (dark-treated) or exposed to light from two GE 30 W, 115 V reflector flood lamps 18 cm distant for 30 min (light-treated). After light or dark treatment, the entire shoot was homogenized in pH 8.9 Tris-glycine electrophoresis buffer, the resultant extract centrifuged 15 min at 20,000 *g*, and 100 μl of the supernatant solution, made 4% in sucrose, 0.005% in bromphenol blue, layered directly onto 7.5% acrylamide gels. Buffer and gels were prepared, and electrophoresis conducted, essentially as described by Gabriel [10] with some modifications. After electrophoresis the gels were immersed in 2 ml of a solution containing 5 μmoles fructose-1, 6-diP, 8.5 μmoles Na_3AsO_4, 50 μmoles pH 7.4 HEPES buffer, 5 enzyme units each aldolase and triose-P isomerase, 0.05 mg phenazine methosulfate, 0.3 mg nitro blue tetrazolium, and 10 μmoles TPN. Gels were scanned at 540 nm using a Gilford 2400 spectrophotometer equipped with a linear transport attachment. Area associated with each peak was measured with a Gelman planimeter.

Gels of different acrylamide concentration for Hedrick–Smith analysis [11] were prepared as described above except that the levels of acrylamide and *N,N'*-methylene bisacrylamide were varied (the ratio of the two acrylamide components was held constant).

3. Results and discussion

Two bands of TPN-linked glyceraldehyde-3-P dehydrogenase activity with relative mobilities 0.16 and 0.21 were found when extracts of light- or dark-treated pea plants were subjected to electrophoresis on

* Part of this study will be included in a thesis which will be submitted to the Graduate School, University of Illinois at Chicago Circle, in partial fulfillment of the requirements for the degree of Master of Science.

Table 1
Glyceraldehyde-3-P dehydrogenase activity associated with fast and slow moving bands after light or dark treatment.

Dark-DPN		Light-DPN		Dark-TPN		Light-TPN		Dithiothreitol TPN or DPN	
Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast
36	64	23	77	44	55	25	75		100
34	65	36	64	43	57	25	75		
46	57	23	77	43	57	24	76		
34	66	24	76	42	58	29	71		
		19	81						
		32	67						
		28	72						
37	63	26	73	43	57	25	74		100

Values are relative to 100. For details see text.

7.5% polyacrylamide gels. Two bands of DPN-linked glyceraldehyde-3-P dehydrogenase activity, coincident with the TPN-linked activity, and occasionally a separate and distinct third band (relative mobility 0.18) were found when DPN replaced TPN in the reaction mixture. This DPN-specific activity band was never observed when the plants had been light-treated.

Following dark-treatment 57% of the TPN-linked enzyme activity was associated with the faster-moving band, and 63% of the DPN-linked activity (table 1). After light-treatment, 74% of the TPN-linked activity was associated with the faster-moving band, and 73% of the DPN-linked activity. This amounts to a 17% relative increase in the percent of activity associated with the faster-moving band in the case of TPN-linked activity, and a 10% increase in the case of DPN-linked activity.

After treatment with 50 mM dithiothreitol all of the dehydrogenase activity, regardless of nucleotide substrate or pretreatment, was associated with the faster-moving band (table 1). Because of the nature of the assays it was not possible to measure absolute enzyme activity, but activity bands always appeared first and were most intense when the extract had been treated with this reducing agent.

The light-dependent increase in the relative amount of glyceraldehyde-3-P dehydrogenase activity associated with the faster-moving band could indicate either that upon illumination some of the activity associated with the slower-moving band is converted to a more active, faster-moving, form of the enzyme, or, that light activates the enzyme associated with the faster-moving band. The fact that treatment with the strong reducing agent dithiothreitol causes a total shift of activity into the faster-moving band strongly suggests that light-dependent conversion of one form to the other does indeed occur, and that this conversion involves light-dependent reduction of disulfide bonds.

The differences in electrophoretic mobility could be related to differences in conformation and charge, or to differences in the state of aggregation of the enzyme. Fig. 1 shows that when log relative mobility in gels of differing porosity is plotted against acrylamide gel concentration for the fast- and slow-moving activity bands, parallel lines are obtained, indicating that the two forms have the same molecular size. The change in electrophoretic mobility must then be the result of a change in net charge on the molecule, presumably resulting from change in conformation brought about by reduction of the enzyme protein.

Although Müller [12] obtained marked stimulation of TPN-dependent glyceraldehyde-3-P dehydrogenase activity in spinach chloroplast extracts at 22° with TPNH or ATP, we were unable to mimic the light effect by treating pea leaf extract with either 17 mM ATP or 3 mM TPNH at 0°. However, we probably would not have been able to observe an enzyme mediated change in the dehydrogenase at the lower temperature.

Hudock et al. [13] reported differences in the number of free SH groups and Michaelis constants for certain substrates for the DPN-linked

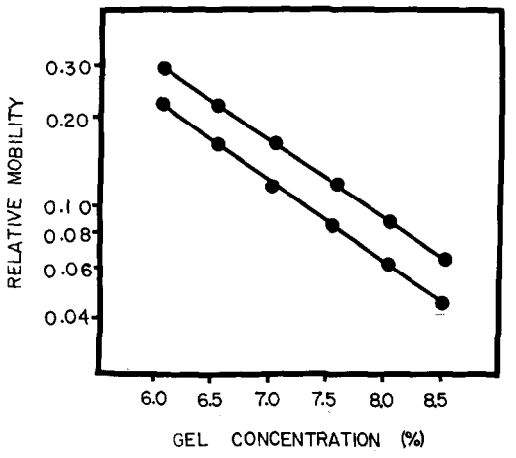

Fig. 1. The effect of different gel concentrations on the mobility of the faster and slower moving forms of TPN-linked glyceraldehyde-3-P dehydrogenase. Parallel lines indicate that the two enzyme forms have similar molecular size but different net charge under the conditions used.

glyceraldehyde-3-P dehydrogenases isolated from photoautotrophically and photoheterotrophically cultured *Chromatium*. The two forms were interconvertable. Apparently the activity of this enzyme in photosynthetic species ranging from the primitive photosynthetic bacteria through higher green plants is regulated by reductive process(es) reflecting environmental conditions.

Some similarities exist between the protein kinase systems operating in animals and this system operating in green plants. Activation of at least three of these light-activated enzymes [3, 6, 7] requires factors which are probably small proteins. It seems possible that an enzyme mediated control system, tied not to cAMP, but to some substance generated upon illumination operates in green plants to control photosynthetic carbon metabolism.

Acknowledgements

We are indebted to Dr. David G. Penney for the loan of most of the equipment used in these experiments and to Mr. James McCorkle and staff for growing the pea plants. This work was supported by a grant, GB 28160, from the National Science Foundation.

References

[1] H. Ziegler and I. Ziegler, Planta 65 (1965) 369.
[2] B.A. Melandri, R. Pupillo and A. Baccarini-Melandri, Biochim. Biophys. Acta 220 (1970) 178.
[3] B.B. Buchanan, P. Schürman and P.P. Kalberer, J. Biol. Chem. 246 (1971) 5952.
[4] E. Latzko, R.v. Garnier and M. Gibbs, Biochem. Biophys. Res. Commun. 39 (1970) 1140.
[5] E. Steiger, I. Zeigler and H. Ziegler, Planta 96 (1971) 109.
[6] G.F. Wildner and R.S. Criddle, Biochem. Biophys. Res. Commun. 37 (1969) 952.
[7] M.D. Hatch and C.R. Slack, Biochem. J. 112 (1969) 549.
[8] H.S. Johnson and M.D. Hatch, Biochem. J. 119 (1970) 273.
[9] K. Lendzian and H. Ziegler, Planta 94 (1970) 27.
[10] O. Gabriel, in: Methods in Enzymology, Vol. 22, ed. W.B. Jakoby (Academic Press, New York, 1971) p. 565.
[11] J.L. Hedrick and A.L. Smith, Arch. Biochem. Biophys. 126 (1968) 155.
[12] B. Müller, Biochim. Biophys. Acta 205 (1970) 102.
[13] G.A. Hudock, D.B. Mellin and R.C. Fuller, Science 150 (1965) 776.