PHOTOSYNTHETIC ELECTRON-TRANSPORT SYSTEM CONTROLS CYTOPLASMIC GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY IN PEA LEAVES

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1. Introduction

There are two forms of glucose-6-phosphate dehydrogenase in the pea (*Pisum sativum* L) leaf. Both are inactivated in vivo when the plant is irradiated [1]. A vicinal dithiol-containing, thylakoid membrane-bound light-effect mediator (LEM) appears to be involved in inactivation of chloroplast glucose-6-dehydrogenase. This mediator is reduced by a component of the electron-transport system located on the reducing side of photosystem I prior to ferredoxin [2,3]. The LEM system also participates in the activation of at least four enzymes of photosynthetic carbon metabolism within the chloroplast [2,4]. Light-modulation of the activity of the chloroplastic enzymes appears to involve thiol, disulfide group exchange ('reshuffling') catalyzed by the reductively activated LEMs [5]. We now wish to report that light-modulation of the activity of the cytoplasmic glucose-6-P dehydrogenase is 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU)-sensitive. These results imply that the photochemical apparatus, rather than a cytoplasmic light acceptor, is involved in the inactivation of the cytoplasmic form of this enzyme.

2. Materials and methods

Pea (*Pisum sativum* L., var. Little Marvel) plants grown 9–12 days in vermiculite under natural light, in a greenhouse, were used in the experiments reported here. Plants or leaves were treated with DCMU, irradiated with white light, then analyzed for cytoplasmic and chloroplastic glucose-6-P dehydrogenase activity using gel electrophoresis [1].

In experiment I (table 1) intact seedlings (9–12) were transplanted into small containers, sprayed with 10^{-4} M DCMU in 0.26 M ethanol or 0.26 M ethanol only, as indicated, held in darkness overnight, then sprayed once more and exposed to 800 foot-candles white-light (two General Electric 30 W, 115 V reflector flood lamps, 18 cm distant) for 15 min. Experiments were run at room temperature (about 22°C). Excess heat from the lights was dissipated with a fan. Dark control plants were not exposed to light. Leaves were removed, ground in electrophoresis buffer (chilled mortar and pestle), debris removed by centrifugation (12 000 × *g*, 10 min) and the two forms of the enzyme separated by electrophoresis on 7–1/2% polyacrylamide gels (see ref. [1]). Specific activity of glucose-6-P dehydrogenase in crude extracts was determined using assay methods described previously [1].

In experiment II plants were held overnight in darkness, leaves were removed into 25 ml thick-walled Erlenmeyer flasks and vacuum infiltrated with 10^{-4} M DCMU in 0.25 M methanol, or with 0.25 M methanol only, as indicated. After an additional 30 min dark-incubation period, the leaves were exposed to 1000 foot-candles white-light (115 V, 40 W, GEF bulb). The infiltration and dark- and light-incubations were carried out at 20°C on a reciprocating shaker (100 strokes/min). After 15 min in the light the leaves were removed and extract prepared and subjected to electrophoresis as in experiment I.

Experiment III was conducted as was experiment II, except that plants were not held in dark overnight and leaves were vacuum-infiltrated in the light.

DCMU used in these experiments was a product of Pfaltz and Bauer, Inc. It contained 50% 3-(*p*-chloro-

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Table 1
Effect of DCMU on light inactivation of cytoplasmic glucose-6-P dehydrogenase

Experiment	Specific activity, crude extract (nmol NADPH formed min^{-1} mg $protein^{-1}$)			Relative activity of cytoplasmic enzyme[a]			DCMU inhibition of light inactivation of cytoplasmic enzyme (%)
	Dark	Light	Light,DCMU	Dark	Light	Light,DCMU	
I	44	39	42	86	74	82	67
II	37	26	39	89	56	74	54
III	30	22	30	67	48	61	68

[a] Based on (%) activity associated with cytoplasmic activity band on gels and setting total activity in dark control at 100
For experimental details see text. Several gels were run/sample and the results averaged. Similar results were obtained in additional experiments in each case.

phenyl)-1,1-dimethyl urea which was corrected for in making up DCMU solutions. DCMU used for infiltration experiments was recrystallized from ethanol prior to use. Biochemicals were obtained from Sigma Chemical Co. All other reagents were analytical reagent grade. Pea seeds were obtained from Northrup and King Seed Company, Chicago.

3. Results and discussion

The experimental results in table 1 indicate that the photosynthetic electron-transport specific-inhibitor DCMU [6] inhibits light-inactivation of cytoplasmic glucose-6-P dehydrogenase. Clearly the photochemical apparatus is involved in the inactivation of the cytoplasmic as well as the chloroplastic enzyme. In the present experiments a wide variation in the relative inhibition of the two forms of the enzyme was observed. This is probably not surprising since light-modulation of the chloroplastic form of the enzyme appears to involve a thylakoid-bound LEM and it seems most unlikely that the cytoplasmic form can interact directly with a chloroplastic LEM. There must then be some other LEM system for modulation of the activity of the cytoplasmic forms of this enzyme and of P-fructokinase [7]. Schürmann et al. [8] have purified a soluble protein, 'ARP_b', which affects the activity of several of the light-modulated enzymes. (All of the evidence in this laboratory speaks against the participation of a soluble mediating enzyme in light-modulation within the chloroplast, but we have not yet demonstrated modulation in a stroma-free system.) Buchanan and Wolosiuk [9] have found ARP_b activity in a variety of organisms and tissues ranging from photosynthetic bacteria through mammalian liver. In view of the ubiquitous distribution of this activity and the high levels reported in seeds, roots and etiolated seedlings [9], it seems possible that a cytoplasmic ARP_b catalyzes thiol, disulfide group-exchange reactions which inactivate the two light-modulated cytoplasmic enzymes. On the other hand, the chloroplast changes size and shape upon illumination [10]. Perhaps there is a LEM embedded in the outer membrane of the chloroplast envelope which controls the activity of the cytoplasmic enzymes. Alternatively, a small molecule effector may be released from the illuminated chloroplast into the surrounding cytosol. Experiments designed to test these and other alternative possibilities are in progress.

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