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Short Communication

Light Modulation of the Activity of Carbon Metabolism Enzymes in the Crassulacean Acid Metabolism Plant *Kalanchoë*[1]

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ABSTRACT

When intact *Kalanchoë* plants are illuminated NADP-linked malic dehydrogenase and three enzymes of the reductive pentose phosphate pathway, ribulose-5-phosphate kinase, NADP-linked glyceraldehyde-3-phosphate dehydrogenase, and sedoheptulose-1,7-diphosphate phosphatase, are activated. In crude extracts these enzymes are activated by dithiothreitol treatment. Light or dithiothreitol treatment does not inactivate the oxidative pentose phosphate pathway enzyme glucose-6-phosphate dehydrogenase. Likewise, neither light, *in vivo*, nor dithiothreitol, *in vitro*, affects fructose-1,6-diphosphate phosphatase. Apparently the potential for modulation of enzyme activity by the reductively activated light effect mediator system exists in Crassulacean acid metabolism plants, but some enzymes which are light-dark-modulated in the pea plant are not in *Kalanchoë*.

Crassulacean acid metabolism plants were long regarded as photosynthetic curiosities because of their peculiar ability to fix CO_2 at night and to evolve CO_2 during the day. Although Vickery and others eventually showed that this CO_2 was actually being fixed into acids, which served as CO_2 reservoirs (20), it was only very recently that Levi and Gibbs (15) reported evidence for the operation of the reductive pentose phosphate pathway in CAM[4] plant chloroplasts.

Light modulation of the activity of the enzymes of photosynthetic carbon metabolism has been shown to occur in C_3 and C_4 plants (3, 18) and, in C_3 plants appears to be mediated by a thylakoid membrane-bound light effect mediator (LEM) which is in turn reductively activated by the photosynthetic electron transport system (5, 6). The effect of light on the activity of certain enzymes involved in acid metabolism in CAM plants has been investigated and it is thought that the light receptor is phytochrome. The light effect is complex; the activity of these CAM enzymes oscillates in circadian fashion and, while the phase is set by light, there is not necessarily an immediate response to irradiation (17).

The purpose of the present experiments was to determine whether reductive pentose phosphate cycle enzymes are subject to light modulation in the CAM plant *Kalanchoë*.

MATERIALS AND METHODS

Most of these experiments were done in January and February, 1977. The *Kalanchoë* "Tetra Vulcan" plants were about 20 cm in height and had been grown from "super seedlings" (seedlings about 3 cm in height obtained in plastic strips from George Ball Company, Chicago) in a 1:1:1 perlite-soil-peat mixture which was treated monthly with a 20:20:20 fertilizer. Natural light was supplemented with artificial illumination to give a 16-hr day (to prevent flowering). Greenhouse temperature was 32 C during the day, 18 C at night. These plants did take up CO_2 in dark and did release CO_2 when illuminated.

All of the plants used in these experiments were placed in the dark for 2 hr before light treatment and/or extract preparation. Subsequent manipulation of dark-treated plants was in darkness or very dim light. Leaf halves (15–20) were cut longitudinally from the dark-treated plants. These were used for preparing the dark-treated control extract. The remaining leaf halves were exposed to white light (480 ft-c) from two General Electric 30-w, 120-v reflector flood lamps, 15 cm distant, for 30 min. The half-leaves were then removed from the plant, the midrib was discarded, and extract was prepared, under the white light source.

Leaves or leaf halves were washed twice in distilled H_2O, then blotted dry on filter paper. Extracts were prepared by grinding tissue in 4 ml of 2% PEG, 10 mM Tris-HCl (pH 8.1) with a chilled mortar and pestle. (PEG at this concentration was absolutely necessary for optimal enzyme activity and high protein solubility. We assume that the PEG prevented precipitation of proteins by tannins.) The extract was centrifuged for 20 min at 15,000*g*. DTT treatment consisted of adding DTT to the supernatant solution to give a concentration of 50 mM and then allowing the solution to stand on ice for at least 1 hr before assaying enzyme activity.

Glucose-6-P dehydrogenase, NADP-linked glyceraldehyde-3-P dehydrogenase, and NADP-linked malic dehydrogenase were assayed as described by Duggan and Anderson (11), as was ribulose-5-P kinase except that the ATP concentration was 1 mM. Fructose-1,6-diP phosphatase was assayed by the method of Preiss and Greenberg (16) at pH 8.3 and without albumin. Change in A_{340} was followed on a Gilford 2400 recording spectrophotometer. Sedoheptulose-1,7-diP phosphatase activity was assayed by the method of Anderson (1). Controls were run in all cases without the carbon substrates and, in the case of the kinase, an additional control was run without ATP.

Protein was precipitated from the extracts with acetone and estimated by the biuret method (4).

Biochemicals and coupling enzymes were purchased from Sigma. PEG was obtained from General Biochemicals and was used without further purification. Other chemicals were highest grade commercially available.

Voucher specimens of the *Kalanchoë* plants used in these ex-

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[4] Abbreviations: CAM: Crassulacean acid metabolism; LEM: light effect mediator.

periments have been deposited in the University of Illinois, Chicago Circle, Herbarium.

RESULTS AND DISCUSSION

There is good reason to believe that the conventional C_3 pathway operates in CAM plants. Levi and Gibbs (15) have reported that CO_2 is fixed into the usual products of the reductive pentose phosphate cycle in *Kalanchoë*. Most of the enzymes of the cycle have been detected in CAM plants. We have now shown that three of these enzymes, NADP-linked glyceraldehyde-3-P dehydrogenase, ribulose-5-P kinase, and sedoheptulose-1,7-diP phosphatase are light-activated in *Kalanchoë*. The activity of each of these enzymes is enhanced when DTT is added to crude whole leaf extracts of this plant (Table I).

Glyceraldehyde-3-P dehydrogenase has been shown to be light-activated in a number of plants including the C_3 plants pea (7), spinach, broad bean, tobacco, oat, the C_4 plants maize and sugar cane (18), and now in CAM plants. Ribulose-5-P kinase, also, has been shown to be light-activated in a number of C_3 plants, but in only one of several C_4 species which have been studied (18). The enzyme is light-activated in the procaryotic blue green alga *Anacystis nidulans,* but not in the photosynthetic bacterium *Rhodospirillum rubrum* (11). As far as we are aware this enzyme has not previously been shown to be present in CAM plants.

Sedoheptulose-1,7-diP phosphatase is light-activated in pea (1) and spinach chloroplasts (10). The enzyme is not light-modulated in *Anacystis* (11) and has not been studied in C_4 species. This is the first demonstration of activity of this reductive pentose phosphate-specific enzyme in CAM plants.

Fructose-1,6-diP phosphatase was not activated when *Kalanchoë* plants were irradiated, nor was the enzyme in crude extracts affected by DTT treatment. To our knowledge this enzyme has only been shown to be light-activated in pea (J. X. Duggan, unpublished) and spinach chloroplasts (10, 13) and extracts (9) although the diurnal variation in the activity of this enzyme noted in tapioca (19) is probably the result of light activation.

Table I. Effect of Light-treatment on Enzyme Activity Levels in Kalanchoë Plants and of DTT-treatment on Activity Levels in Leaf Extracts.

Plants were dark-treated for two hours before irradiation (480 ft-c white light) and/or preparation of extracts. Values given are means of 3 activity determinations. Each line represents a separate experiment with respect to enzyme assayed. Activity is expressed as nmoles substrate consumed or product formed min^{-1} mg $prot^{-1}$.

Enzyme	Treatment			Stimulation X-fold	
	Dark	Light	DTT	Light	DTT
Ribulose-5-P Kinase	41	250		6.1	
	42	110		2.6	
	46	67		1.5	
	60		130		2.2
NADP-linked Glyceraldehyde-3-P Dehydrogenase	50	103		2	
	23	44		2	
	75		170		2.3
	52		130		2.5
Sedoheptulose-1,7-diPase	0.56	0.96		1.7	
	0.67	1.04		1.6	
	0.38		0.96		2.5
	0.19		0.52		2.7
Fructose-1,6-diPase[a]	31	32			
	10	12			
	6	6			
	11		12		
	13		13		
NADP-linked Malic Dehydrogenase	33	68		2.1	
	72	110		1.5	
	87	140		1.6	
	10		340		34
	40		810		20
Glucose-6-P Dehydrogenase	7	7.8			
	15	17			
	11		13		
	13		12.8		

[a]Assay pH was 8.3. No activation was found when assay pH was 8.0.

Why is light modulation of fructose-1,6-diP phosphatase activity advantageous to the C_3 plant but not to the CAM plant? We reason that if in the CAM plant, as in the C_3 plant, the triose phosphates and P-glycerate, but not hexose phosphates, can be transported in and out of the chloroplast, and if, in the CAM plant, the three carbon units formed from decarboxylation of four carbon acids must be converted back into starch during the daylight hours, then the CAM plant chloroplastic fructose-1,6-diphosphatase must have two major functions. The first is to participate in the reductive pentose phosphate cycle in the reductive assimilation of CO_2, as in C_3 plants. The second is to participate in the Embden-Meyerhof-Parnas pathway in the reformation of hexose-P from the three carbon units formed when the four carbon acids are decarboxylated. These plants do evolve CO_2 in sunlight. If there is an excess of CO_2 then there must also be an excess of three carbon pieces which must be converted back to starch. If this conversion must proceed even when photosynthesis does not and if CAM chloroplast fructose-1,6-diP phosphatase is bifunctional and does participate in both of these pathways, then control of the enzyme by the LEM system will not be advantageous. Our experiments do not eliminate the possibility of temporal control of fructose-diphosphatase activity, nor do they eliminate light as the phase setter for such control.

The NADPH-generating enzyme, glucose-6-P dehydrogenase, is not inactivated when *Kalanchoë* plants are irradiated. In crude leaf extracts the enzyme is not affected by DTT treatment (Table I). Both the chloroplast and cytoplasmic forms of this enzyme in the pea leaf are light-modulated (2); the photochemical apparatus appears to be involved in inactivation of the cytoplasmic, as well as the chloroplastic form (8).

This enzyme may be involved in maintaining reducing potential in the dark in C_3 and C_4 plants. If there are other means for controlling reductant levels in CAM plants, dark activation of this dehydrogenase might be supererogatory.

NADP-linked malic dehydrogenase in leaves of *Kalanchoë* is light-activated, but only about 1.7-fold (Table I). This stimulation is considerably lower than was observed in experiments with the C_3 plants pea (5), spinach (15) and barley (18), or with the C_4 plant maize (12). The difference in this case lies in the higher levels of activity in the dark treated *Kalanchoë*, rather than in the levels of activity in irradiated tissue. In C_3 plants dark NADP-linked malic dehydrogenase levels are nil (5, 12); in contrast in CAM plants dark levels appear to be high (Table I; ref. 14).

These experiments demonstrate that light-dark modulation of the activity of the enzymes of photosynthetic carbon metabolism does occur in CAM plants as well as in C_3 and C_4 plants.

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