SUBSTRATES AND INORGANIC PHOSPHATE CONTROL: THE LIGHT ACTIVATION OF NADP-GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE AND PHOSPHORIBULOKINASE IN BARLEY (*HORDEUM VULGARE*) CHLOROPLASTS*

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1. Introduction

Mesophyll chloroplasts of C_3 plants contain several photosynthetic enzymes that are light activated [1–5]. Light modulation of enzyme activity appears to involve formation of membrane-bound vicinal dithiols by photosynthetic electron transport [2] and certain soluble stromal proteins that have been tentatively identified as thioredoxin and ferredoxin-thioredoxin reductase [5]. In the case of the enzyme NADP-glyceraldehyde-3-*P* dehydrogenase, metabolites such as ATP, NADPH, and Pi also cause activation [6]. It is thought that this effector-medicated control operates in conjunction with reductive control mechanisms.

Evidence is presented in this paper that photosynthetic intermediates and Pi modulate the light activation of at least two photosynthetic enzymes in intact barley chloroplasts. In general, conditions that increased the induction phase of CO_2-dependent O_2 evolution decreased the velocity of enzyme activation. Addition of substrates (e.g., PGA) to intact chloroplasts decreased the induction phase of O_2 evolution and increased the velocity of enzyme activation. Enzyme activation in broken chloroplast preparation was inhibited by Pi. In this system, substrates were not required for light activation, but reversed the inhibition by Pi. The results suggest that the chloroplast substrate/Pi ratio may modulate enzyme activation in the light.

Abbreviations: Pi, inorganic phosphate; PGA, 3-phosphoglycerate; R5P, ribose-5-phosphate; Chl, chlorophyll

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2. Materials and methods**

2.1. *Mesophyll chloroplast isolation*

Mesophyll protoplasts were isolated from 6- to 12-day leaves of barley (*Hordeum vulgare* L. cv. Trophy) as previously described [7]. Intact (93–99%) chloroplasts were prepared by mechanically rupturing protoplasts in 0.3 M sorbitol, 5 mM $MgCl_2$, 10 mM $Na_4P_2O_7$, and 2 mM isoascorbate (pH 6.5). After centrifugation (200 × *g*, 3 min), the chloroplast pellet was resuspended in 0.3 M sorbitol, 50 mM HEPES–NaOH (pH 7.6), 1 mM $MgCl_2$, 1 mM $MnCl_2$, and 2 mM EDTA (resuspension medium).

2.2. O_2 *evolution*

O_2 evolution was followed polarographically at 25°C. The reaction mixture contained 0.3 M sorbitol, 50 mM Tricine–NaOH (pH 8.2), 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA, 0.2 mM Na_2HPO_4, 6 mM

$NaHCO_3$, and 20–40 μg Chl/ml. Illumination was provided by a 75-W floodlamp giving a total quantum flux density of 80 nE/cm^2·s between 400 and 700 nm at the face of the cuvette.

2.3. *Enzyme assays*

NADP-glyceraldehyde-3-phosphate dehydrogenase was assayed using the method of Latzko and Gibbs [8]. The assay medium for phosphoribulokinase contained 100 mM Tricine–NaOH (pH 8.0), 10 mM $MgCl_2$, 2 mM ATP, 5 mM phosphoenolpyruvate, 0.4 mM NADH, 0.5 mM R5P, 6 U/ml pyruvate kinase, 9 U/ml lactate dehydrogenase and 1 U/ml phosphoribuloisomerase.

2.4. *Light activation*

Intact or broken chloroplast preparations were incubated in the light (approximately 80 nE/cm^2·s) at 25°C. At the indicated times (see text), aliquots were removed and diluted 5-fold with ice-cold redistilled H_2O. Assays were performed with the resultant extract. Intact chloroplasts were incubated in the same medium used to measure O_2 evolution. Broken chloroplast preparations were obtained by resuspending a chloroplast pellet (200–300 μg Chl) in 800 μl of one-tenth dilution of resuspension medium. After centrifugation (5000 × *g*, 5 min), the supernatant (2–3 mg proteins) was removed. As indicated in the text, the supernatant was desalted by passage through a Sephadex G-25 column. The pellet was resuspended in 500-μl full-strength resuspension medium. Thylakoid membranes (130–200 μg Chl/ml) and stromal proteins (1600–2500 μg protein/ml) were mixed and incubated as described above.

2.5. *Chlorophyll and protein determination*

Chlorophyll was determined in 96% ethanol using the extinction coefficients of Wintermanns and De Mots [9]. Protein was determined by the Lowry method [10].

3. Results and discussion

3.1. *Light activation in intact chloroplasts*

When intact barley chloroplasts were illuminated with CO_2 as sole substrate, O_2 evolution began gradually after an induction phase of approximately

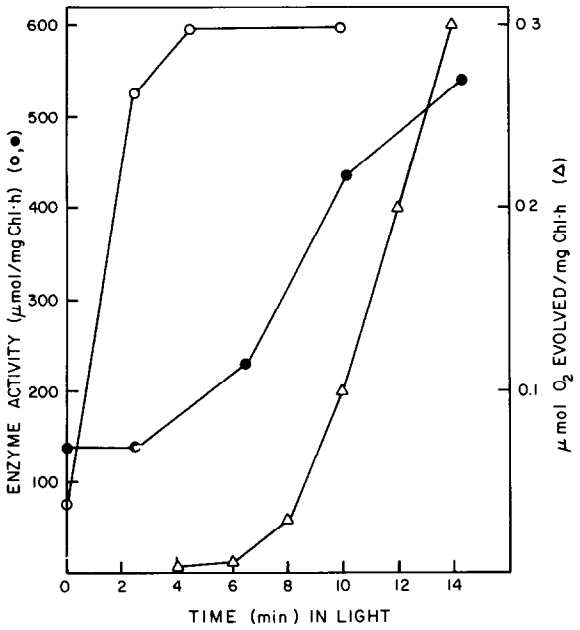

Fig.1. Time course of light activation of phosphoribulokinase (○), NADP-glyceraldehyde-3-*P* dehydrogenase (●), and O_2 evolution (△) in intact barley chloroplasts.

8 min (fig.1). Before the onset of O_2 evolution there was a marked increase in activity of NADP-glyceraldehyde-3-*P* dehydrogenase and phosphoribulokinase (fig.1). Light activation of enzyme activity has been demonstrated previously with intact pea [2] and spinach [3,4] chloroplasts. As shown in fig.1, the velocity of activation for the enzymes was quite different. Phosphoribulokinase was completely activated after 4 min in the light, whereas complete activation of NADP-glyceraldehyde-3-*P* dehydrogenase required more than 14 min in the light. In this and other experiments (data not shown), the increase in NADP-glyceraldehyde-3-*P* dehydrogenase activity paralleled and slightly preceded the evolution of O_2. The results suggest that the two processes are related.

The length of the induction phase of O_2 evolution in intact chloroplasts can be increased by addition of Pi and decreased by addition of PGA [11]. Increasing the concentration of Pi decreased the velocity of NADP-glyceraldehyde-3-*P* dehydrogenase activation in intact barley chloroplasts. Addition of 0.5 mM Pi increased the induction phase of CO_2-dependent O_2 evolution from 3.5 to 10 min (data not shown) and

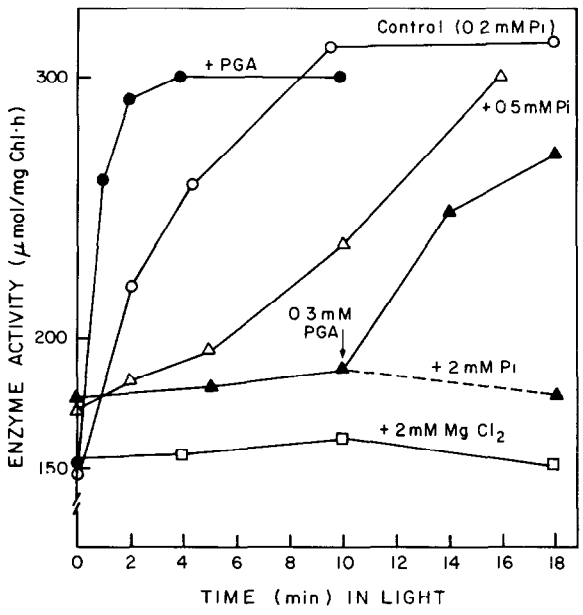

Fig.2. Time course of light activation of NADP-glyceraldehyde-3-*P* dehydrogenase in intact barley chloroplasts. Additions to the basic reaction medium were as indicated.

significantly increased the time required to fully activate the enzyme (fig.2). Two millimolar Pi completely prevented O_2 evolution (i.e., infinite induction phase) and the light activation of enzyme activity (fig.2). The inhibition by Pi of O_2 evolution and enzyme activation was reversed by PGA. Addition of 0.3 mM PGA to intact chloroplasts inhibited by 2 mM Pi resulted in a rapid increase in dehydrogenase activity (fig.2) which was accompanied by an increase in O_2 evolution from 0 to 45 μmol O_2/mg Chl·h (data not shown). In the presence of 0.2 mM Pi (optimum for O_2 evolution), PGA decreased the induction phase of O_2 evolution from 3.5 to 0.7 min (data not shown) and significantly increased the velocity of enzyme activation in the light (fig.2). In general, conditions that shortened the induction phase of O_2 evolution increased the velocity of enzyme activation. Similarly, conditions that increased the induction phase decreased the velocity of enzyme activation. Concentrations of $MgCl_2$ and Pi that completely blocked O_2 evolution (2 mM; data not shown) completely prevented the increase in enzyme activity by light (fig.2).

Magnesium inhibition of CO_2-dependent O_2 evolution by isolated chloroplasts [12,13] has been well documented. It has been proposed that magnesium inhibits O_2 evolution by preventing the light-activation of photosynthetic enzymes [14]. This was confirmed by the results shown in fig.2. It has been further proposed [14] that magnesium enhances Pi-exchange across the chloroplast envelope and that depletion of chloroplast substrates then prevents the light-activation process. In order to test the hypothesis, the light-activation of enzymes was studied in broken chloroplast preparations to avoid restraints imposed by the chloroplast envelope.

3.2. *Light activation in broken chloroplast preparations*

Illumination of a broken chloroplast preparation (reconstituted mixture of thylakoid membranes plus stromal proteins) resulted in a 1.8-fold stimulation of NADP-glyceraldehyde-3-*P* dehydrogenase and phosphoribulokinase activity (table 1). The increase in activity was not inhibited by 5 mM $MgCl_2$, but was blocked by 2 mM Pi (table 1). With intact chloroplasts, light-activation was prevented by both magnesium and Pi (fig.2). The results are consistent with the proposal that magnesium inhibits light activation of enzymes in intact chloroplasts by activating Pi-exchange across the envelope. It appears that Pi, which inhibits light activation in both intact (fig.2) and broken chloroplast (table 1) preparations, is responsible for the inhibition.

Table 1
Effect of magnesium and Pi on the light activation of photosynthetic enzymes in broken chloroplast preparations of barley

Condition	Enzyme (μmol product/mg Chl·h)	
	NADP-glyceraldehyde-3-*P* dehydrogenase	Phosphoribulokinase
Dark	252	168
Light[a]	444	288
Light[a] +5 mM $MgCl_2$	426	288
Light[a] +2 mM Pi	246	144

[a] 5-min illumination (maximal light activation)

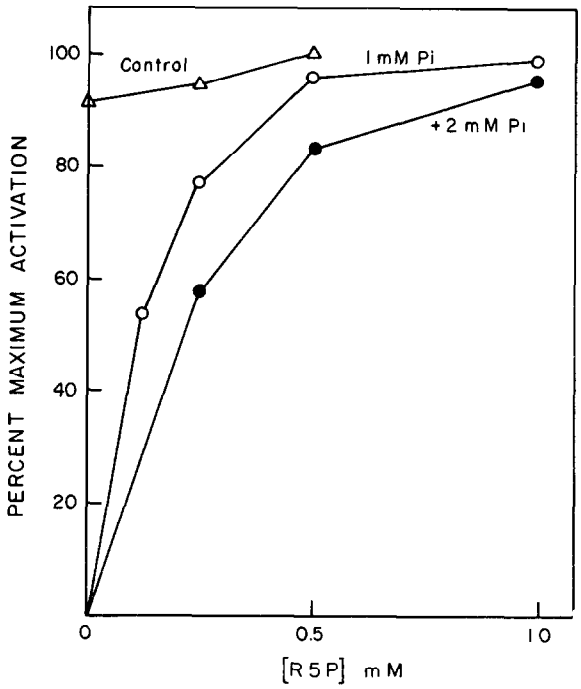

Fig.3. Effect of R5P on light activation of NADP-glyceraldehyde-3-*P* dehydrogenase in broken chloroplast preparations of barley. Maximum light activation (enzyme activity in light minus dark), expressed as μmol product formed/mg Chl·h control, 130; +1 mM Pi, 189; +2 mM Pi, 200.

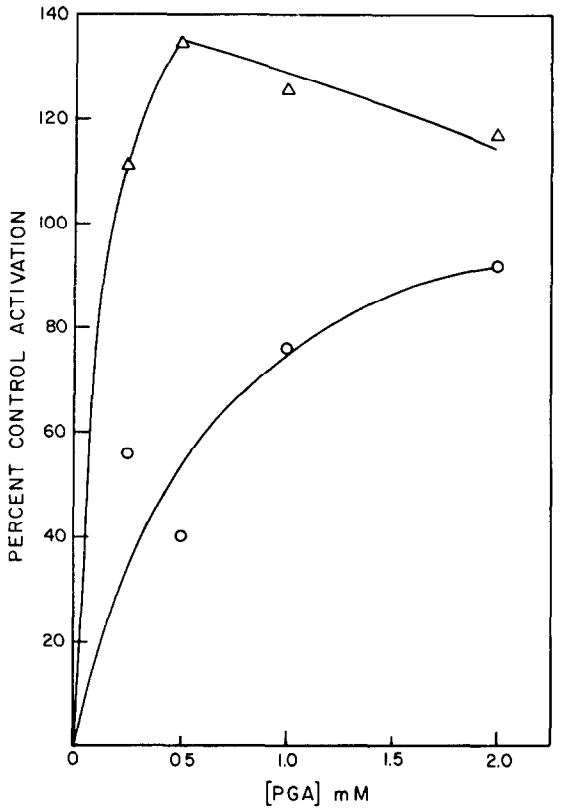

Fig.4. Effect of PGA on light activation of NADP-glyceraldehyde-3-*P* dehydrogenase (○) and phosphoribulokinase (△) in broken chloroplast preparations of barley. All reactions contained 2 mM Pi Light activation in the control (minus Pi) was 160 μmol product formed/mg Chl·h.

Phosphate-inhibition of NADP-glyceraldehyde-3-*P* dehydrogenase light-activation in broken chloroplast preparations was reversed by photosynthetic intermediates. The results presented in fig.3 show that 0.5 mM R5P overcame inhibition by 1 mM Pi, whereas 1.0 mM R5P was required to reverse inhibition of light activation by 2 mM Pi. Concentrations of R5P up to 0.5 mM had no significant effect on light activation in the absence of Pi (fig.3, control).

Phosphoglycerate also reversed Pi-inhibition of light-activation in broken chloroplast preparations. Inhibition of phosphoribulokinase light activation by 2 mM Pi was reversed by 0.25 mM PGA, whereas 2 mM PGA was required to reverse inhibition of NADP-glyceraldehyde-3-*P* dehydrogenase light activation (fig.4).

For the experiments presented in figs.3 and 4, stromal proteins were desalted by passage through a Sephadex G-25 column. Removal of low molecular weight compounds (e.g., Pi, substrates) did not affect the kinetics or maximum extent of enzyme light activation (data not shown), indicating that substrates (e.g., PGA and R5P) are not required for enzyme activation. However, the results presented in fig.3 suggest that light activation may be controlled by the substrate/Pi ratio. Hence, light activation would proceed normally at an optimum substrate/Pi ratio or in the complete absence of substrates and Pi. The results of fig.4 further indicate that the optimum substrate/Pi ratio may vary with different enzymes, an effect which would be consistent with the observation that the velocity of light activation of NADP-glyceraldehyde-3-*P* dehydrogenase was slower than phosphoribulokinase in intact chloroplasts ([2] and

fig.1). The observed time course of enzyme activation in intact chloroplasts may be explained by changes in the stromal concentration of Pi and organic phosphates following the onset of illumination. It has been shown in spinach chloroplasts that the level of internal Pi drops significantly after illumination until it reaches a steady state level [15,16]. The total chloroplast phosphate pool remains constant, because as the inorganic concentration decreases, the organic phosphate concentration increases [15]. Increases in the levels of pentose monophosphates, triose phosphates and PGA upon illumination have been documented [17]. Hence, the substrate/Pi ratio would be expected to increase during the first several minutes of illumination. Therefore, enzymes that require a high substrate/Pi ratio for activation (e.g., NADP-glyceraldehyde-3-*P* dehydrogenase) would be activated after enzymes that require a lower ratio for light-activation (e.g., phosphoribulokinase).

4. Concluding remarks

The results presented in this communication support the proposal that magnesium inhibits the light activation of enzymes in intact chloroplasts by stimulating phosphate-exchange across the envelope [14]. The proposal predicts that whereas both Pi and magnesium prevent light activation in intact chloroplasts, only Pi should inhibit in broken chloroplast preparations. This was observed (fig.2 and table 1).

Results were also obtained in which substrates prevented Pi-inhibition of enzyme light activation in broken chloroplast preparations (figs.3 and 4). Because substrates are not required per se for the light activation process, it appears that the substrate/Pi ratio, rather than absolute concentrations, may modulate the process. This mechanism may function to regulate the pool sizes of photosynthetic intermediates in the light in order to allow maximum Calvin cycle activity.

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