Energetic aspects of the light activation of two chloroplast enzymes: fructose-1,6-bisphosphatase and NADP-malate dehydrogenase

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Abstract. The light energy requirements for photoactivation of two chloroplast enzymes: fructose-1,6-bisphosphatase and NADP-malate dehydrogenase were studied in a reconstituted chloroplast system. This system comprised isolated pea thylakoids, ferredoxin (Fd), ferredoxin-thioredoxin reductase (FTR) thioredoxin m and T (Tdm, Tdf) and the photoactivatable enzyme. Light-saturation curves of the photoactivation process were established with once washed thylakoids which did not require the addition of Td for light activation. They exhibited a plateau at 10 W m⁻² under nitrogen and 50 W m⁻² under air, while NADP photoreduction was saturated at 240 W m⁻². Cyclic and pseudo-cyclic phosphorylations saturated at identical levels as enzyme photoactivations. All these observations suggested that the shift of the light saturation plateau towards higher values under air was due to competing oxygen-dependent reactions. With twice washed thylakoids, which required Td for enzyme light-activation, photophosphorylation was stimulated under N₂ by the addition of the components of the photoactivation system. Its rate increased with increasing Td concentrations, just as did the enzyme photoactivation rate, while varying the target enzyme concentration had only a weak effect. Considering that Td concentrations were in a large excess over target enzyme concentrations, it may be assumed that the observed ATP synthesis was essentially dependent on the rate of Td reduction.

Under air, Fd-dependent pseudo-cyclic photophosphorylation was not stimulated by the addition of the other enzyme photoactivation components, suggesting that an important site of action of O₂ was located at the level of Fd.

Abbreviations

Fd, ferredoxin; FBPase, fructose-1,6-bisphosphatase; FTR, ferredoxin-thioredoxin reductase; LEM, light effect mediator; NADP-MDH, NADP-malate dehydrogenase; Td, thioredoxin.

Introduction

The rapid light regulation of several chloroplast enzymes has been shown to involve thiol modulation catalyzed either by the ferredoxin-thioredoxin system [6] or by the membrane-bound LEM system [1]. Both mechanisms require photosynthetic electron transfer which provides the reducing power needed for the final reduction/isomerisation of disulfide bridges of the inactive enzymes into vicinal dithiols. Recent studies [13, 14, 16] have suggested that FBPase and NADP-MDH light activations are very sensitive
to the redox state of electron carriers, but little is known about the magnitude of the electron transfer needed for enzyme photoactivation, compared to other reducing-power consuming reactions such as NADP photoreduction. The only available data concern experiments with protoplasts and intact chloroplasts [8]. In these experiments, light-saturation of three Calvin-cycle enzymes was shown to be reached at about 40 W·m⁻². However, the intact chloroplast is a rather complicated system in which competitions for electrons may occur between different reductant dependent reactions. We have tried to measure the energy requirement of the photoactivation of either FBPase, a Calvin cycle enzyme, or NADP-MDH, an enzyme believed to function in the malate-oxaloacetate shuttle, in a simplified reconstituted chloroplast system [10, 26] in which competitions with other reactions are minimal. 

This system comprised washed thylakoids, the purified components of the ferredoxin-thioredoxin system and the target enzyme. The energy requirement was measured by establishing light-saturation curves for enzyme photoactivation under nitrogen and under air and comparing them to the light-saturation values for NADP reduction. The energetic aspect of enzyme photoactivation was also investigated by measuring the photophosphorylation induced by the addition of the components of the enzyme photoactivation system.

Material and methods

1. Plant material

Peas (Pisum sativum L. cv Merveille de Kelvedon) were grown in a greenhouse, on vermiculite, and watered daily with tap water. In winter, the natural daylight was supplemented with fluorescent bulbs providing 60 W·m⁻², 15 h per day.

2. Intact chloroplast and thylakoid preparation

Intact chloroplasts were isolated from 10 days-old pea shoots by the method of Nakatani and Barber [21]. Thylakoids were obtained by disrupting washed intact pea chloroplasts in 50 mM HEPES buffer, pH 7.8 containing 5 mM MgCl₂ and washing them once or twice in the same buffer by centrifugation (3000 g, 5 min). The final thylakoid pellet was suspended in a 50 mM HEPES, pH 7.8 containing 330 mM sorbitol and 0.5 mM MgCl₂ to a chlorophyll concentration of ca. 2 mg/ml. Chlorophyll was determined by the method of Arnon [3].

3. Enzyme purification

Ferredoxin was purified from spinach leaves by the method of Mayhew [17]. Thioredoxins and NADP-MDH were purified as described previously [9, 10] either from spinach or from corn. FTR was extracted