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Short Communication

A Rapid Phytochrome-dependent Reduction of Nicotinamide Adenine Dinucleotide Phosphate in Particle Fraction from Etiolated Bean Hypocotyl

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Several different biochemical changes in plant cells are controlled by the phytochrome system (4), and the metabolism related to nicotinamide nucleotide coenzymes had recently been reported to be phytochrome-dependent by some workers (1, 8, 11). Fujii and Kondo (3) observed a red light-activated increase of NADPH level in node tissues of oat. Hitherto, little has been known about the intracellular localization of phytochrome. Gordon and Surrey (6) have suggested that mitochondria contain phytochrome, since the rate of oxidative phosphorylation in mitochondria isolated from rat liver was affected by red and far red irradiations. This result has failed to be confirmed (7).

In 1959, Forti *et al.* (2) reported evidence of the existence, in pea stems, of glucose-6-P oxidizing particles which sedimented at lower centrifugal forces than did true mitochondria, but Ragland and Hacket (10) were unable to find any evidence for special glucose-6-P oxidizing particles that were distinct from typical mitochondria.

The aim of the present investigation, therefore, was to examine the possibility of whether the red and far red reversible effect on the NADPH formation present is detectable in a low speed sedimentary particle fraction prepared from etiolated bean hypocotyl tissues.

MATERIALS AND METHODS

Seeds of *Phaseolus vulgaris* L. var humilis were germinated in vermiculite in a dark room at 26 C. Apical 2 cm long hypocotyls were cut from the seedlings cultured for 5 days by means of scissors, and their cotyledons were removed. Fifty grams of the hypocotyl tissue were ground in a cold mortar with a pestle, adding 0.5 g of Polyclar AT (polyvinyl polypyrrolidone, Gokyo Co., Tokyo) and 100 ml of 10 mM tris HCl buffer, pH 7.2, containing 0.4 M sucrose, 1 mM EDTA, 1 mM dithiothreitol, and 1% potassium dextran sulfate (Meito Co., Nagoya). The homogenate was filtered through two layers of cheesecloth and centrifuged at 1,000*g* for 10 min. The supernatant obtained was further centrifuged at 7,000*g* for 15 min, and the pellet was resuspended in 10 mM tris HCl buffer, pH 7.2, containing 0.4 M sucrose, 1 mM EDTA, and 1% potassium dextran sulfate. The suspension was centrifuged at 500*g* for 10 min, and pellets were discarded. The supernatant was washed once again with the same buffer. Finally, the 1000 to 7000*g* sedimentary particle fraction was suspended into 5 ml of the buffer and was used in the present experiments. All the above procedure was carried out in a dark cold room at 3 C under a dim green safelight. The sources of red, far red, and green safelight were the same as described elsewhere (9).

RESULTS AND DISCUSSION

A time course study of NADP reduction was made in reaction mixtures of the particle fraction to see whether red or far red light affect the activities of particle-bound phytochrome and NADP-linked enzymes. The suspension of 1000 to 7000*g* sedimentary particle fraction was exposed to 800 ergs cm^{-2} sec^{-1} red light for 8 min or 15 kergs cm^{-2} sec^{-1} far red light for 2 min at about 0 C. Then, 0.1 ml of each suspension was pipetted into 1 ml of reaction mixture which consisted of 50 μmoles of tris HCl buffer, pH 7.6, 400 μmoles of sucrose, 0.25 μmoles of NADP, 20 μmoles of $MgCl_2$, and 5 μmoles of glucose-6-P. The reaction mixture was incubated in the dark, shaking reciprocally at 30 C. Two milliliters of 0.1 N KOH were added to the reaction mixture, and it was then boiled for 2 min to stop the reaction and to destroy any NADP remaining (5). After cooling, the mixture was neutralized with 0.1 N HCl and 1 ml of 0.5 M tris HCl buffer, pH 7.6, and 0.5 ml of dichlorophenol indophenol (2 μmoles) was added to the mixture. The assay of NADPH was carried out with glucose-6-P dehydrogenase and NADPH diaphorase by a method modified from that of Yamamoto (12). The results are presented in Figure 1. The amount of NADPH increased rapidly after red irradiation, reaching a maximum within a few minutes, compared with a much slower rise in the far red-treated sample. In a reaction mixture, to which was added the particle fraction boiled at 90 C for 1 min, no detectable NADPH was formed. In the 100,000*g* supernatant fraction, phytochrome and glucose-6-P dehydrogenase were detected, but light-dependent formation of NADPH was not observed.

Next, we found the typical red far red reversible effect on the activity of NADPH-linked glucose-6-P dehydrogenase when the above reaction mixture was exposed to red for 8 min and/or far red light for 2 min (Table I). The results show that the red light-induced stimulation of NADPH formation was reversed to a level equivalent to that produced by far red light,

if the far red was given immediately after the red light, and that a sample irradiated with red light following far red treatment yielded NADPH at a level as high as one treated with red light alone. Thus, the low energy light-dependent changes of NADPH formation in the cell-free system used here are suggestive as being regulated in some way by a phytochrome system.

Since photoreversible changes of absorbance difference between 660 nm and 730 nm ($\Delta(\Delta A)/cm = 4.3 \times 10^{-4}$) was spectrophotometrically detectable with the 1000 to 7000*g* sedimentary particle fraction used in this work, phytochrome must be located somewhere in the particle fraction. The above studied reaction was heat-labile, suggesting that this reaction is probably enzyme-dependent. When glucose-6-P was not added to the reaction mixture, only a little NADPH was formed, indicating that the NADPH formation was dependent mainly upon the activity of glucose-6-P dehydrogenase.

There appear to be several possibilities for explaining the mechanism of phytochrome-dependent apparent activation of glucose-6-P dehydrogenase in the particle fraction. Far red-absorbing form of phytochrome might induce some conformational change of the enzyme which consequently bring about its activity. Or, the phytochrome could activate the membrane transport of NADP into the particle. Further investigation into the phytochrome mechanism of this reaction is now in progress in these laboratories.

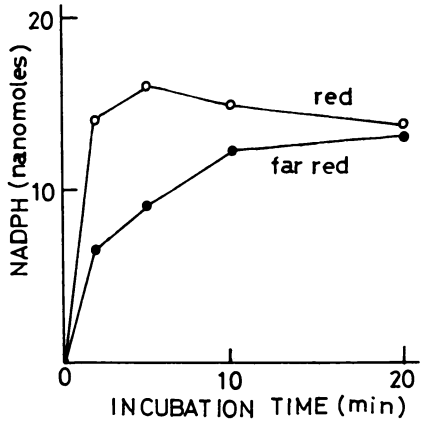

FIG. 1. Time course of the NADPH formation in reaction mixtures composed of particle fractions which were previously exposed to either red light (○) for 8 min or far red light (●) for 2 min.

Table I. *Red and Far Red Reversible Effect on NADPH Formation*

The amount of NADPH in the 1000 to 7000 *g* sedimentary particle fraction was measured at 5 min after the irradiation. Red and/or far red light was exposed for 8 min and 2 min, respectively.

Treatment	NADPH Formed
	nmoles
Dark	10.2
Far red	9.0
Red	15.9
Red/far red	9.2
Far red/red	13.8

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