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PHYTOCHROME MEDIATION OF PEROXIDASE ACTIVITY IN *ZEA MAYS**

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Phytochrome (Pfr) regulates the peroxidase activity in *Zea mays*. Irradiation of etiolated seedlings with red light (660 nm) resulted in an increase in the peroxidase activity. When seedlings were irradiated with red light followed by far-red light, the increase in peroxidase activity was reversed. On exposing dark grown seedlings to continuous far-red light, the enzyme activity increased, indicating an operation of 'high intensity reaction' in *Zea mays*. The mechanism of action of phytochrome in regulating peroxidase activity is discussed.

INTRODUCTION

Light is perhaps the most important environmental factor, which affects living organisms in variety of ways. The morphogenetic effect of light in plants is usually mediated by photoreceptor pigment called phytochrome. Phytochrome is a bluish chromoprotein which exists in two different forms, Pr, absorbing maximally in red region (660 nm), and Pfr, absorbing maximally in far-red region (730 nm). These two forms are interconvertible by absorbing light of appropriate wavelength. Thus, 660 nm light converts Pr to Pfr, and 730 nm light reverts Pfr back to Pr. Out of these two, Pfr is the physiologically active form of phytochrome. Phytochrome affects all phases of plant development such as seed germination, vegetative growth, reproduction and senescence. The molecular mechanism by which phytochrome controls such diverse array of photoresponses has yet to be elucidated. Nevertheless, one of the pertinent actions of phytochrome is the modulation of activity of certain enzymes in plants either through *de novo* synthesis (Acton, Drumm & Mohr, 1974; Attridge, 1974) or by activation of pre-existing inactive enzyme molecules (Attridge, Johnson & Smith, 1974).

The kinetics of enzyme activity under the influence of phytochrome can be resolved into two categories, viz., photomodulation and photodetermination (Schopfer, 1973). In case of photomodulation, the onset and progress of photoresponse depends on continuous presence of Pfr in the system, while in photodetermination, photoresponse once set in by Pfr, can continue even in its absence. In this paper, we present evidence, which suggests that enhancement in peroxidese activity in maize by phytochrome is regulated in a manner characteristic of photomodulation, and that the mechanism of enhancement can be interpreted in terms of phytochrome mediated *de novo* synthesis of the enzyme.

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Materials and Methods

Six-day-old etiolated seedlings of maize (*Zea mays*) were irradiated either with red light (500 μw $cm^{-2}sec^{-1}$) or far-red light (140 μw cm^{-2} sec^{-1}). Enzyme was extracted by grinding apical leaves in 2 ml of 0.1 M phosphate buffer, *p*H 7.0 with 50 mg of insoluble polyvinylpolypyrrolidone at 4°C. The resulting homogenate was centrifuged at 26,000 x g for 20 min at 2°C, and the supernatant was used for enzyme assay and protein determination. Methods employed here have been described in detail elsewhere (Sharma, 1974).

Results and Discussion

Peroxidase is one of the enzymes, which is thought to play a key role in plant development and differentiation, although, its precise function is little understood (Galston & Davies, 1969) Although, the activity of peroxidase has been shown to be modulated under the influence of various growth regulators and environmental factors, yet the role of light in regulation of peroxidase activity has hardly been investigated. An enhancement in peroxidase activity was observed on transferring etiolated detached leaves of maize to light, but the involvement of phytochrome in this response was not studied (Graham *et. al.*, 1970). In present investigation, a brief irradiation of red light to etiolated maize seedlings, which lead to an enhancement in peroxidase activity was fully negated if subsequently followed by far-red light (Table I) which suggests involvement of phytochrome in regulating peroxidase activity in maize. The reversibility of red-light mediated response by far-red light fulfils the essential operational criterion for the involvement of phytochrome in a photoresponse (Mohr, 1972). A significant enhancement in peroxidase activity under continuous far-red light demonstrates the operation of high intensity reaction, which is considered to be mediated via phytochrome by maintaining a low and steady state level of Pfr (Hartmann, 1966).

Table I

Effect of brief irradiation of red and far-red light on the peroxidase activity in apical leaves of maize seedlings

Treatment	Relative enzyme activity (%)
7 days d	100
6 days d+5 min R+24 h d	162
6 days d+5 min FR+24 h d	121
6 days d+5 min R+5 min FR+24 h d	123
6 days d+24 h FR	183

D, Dark; *R*, Red light; *FR*, Far-red light.

Kinetics of peroxidase activity under continuous far-red light showed that after a lag phase of 2 hr, the activity increased steadily till 20 hr (Fig. 1). However, when far-red irradiation was terminated after 12 hr, the peroxidase activity dropped instantaneously. On the other hand, if 6.5 day-old etiolated seedlings were transferred to far-red light, the activity increased after a lag phase of 2 hr. The decline in the rate of enhancement of

peroxidase activity with the termination of far-red light, which would invariably lead to a decline in the level of Pfr, demonstrate the continuous requirement of Pfr in the system to maintain the steady rate of enhancement–a characteristic essential of photomodulation. In contrast to our results, in *Sinapis alba,* it has been found that the enhancement in peroxidase activity once potentiated by Pfr did not require continuous presence of Pfr in the system (Schopfer & Pachy, 1973). Thus, the mode of regulation of the same enzyme by phytochrome in two different systems appears to be different.

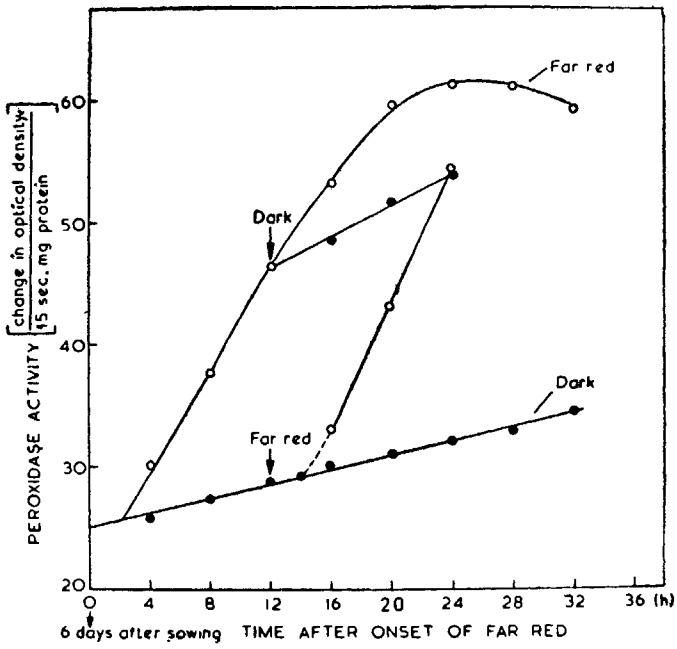

FIG. 1. Effect of continuous FR irradiation on peroxidase activity in *Zea mays*. The arrows indicate the time, when the seedlings were transferred from dark to FR (O—O) and FR to dark (●—●)

To study the mechanism of phytochrome-mediated enhancement in peroxidase activity, effects of various inhibitors of RNA and protein metabolism were studied. Results are shown in Table II. It was found that actinomycin-D (8 μg/ml) and D-*threo* chloramphenicol (50 and 250 μg/ml) had no singnificant effect on the enzyme activity thereby, excluding any possibilty of a transcriptional control or role of organelle ribosomes in phytochrome mediated enhancement in peroxidase activity. On the other hand, puromycin (100 μg/ml) completely nullified the enhancement in peroxidase activity suggesting *de novo* synthesis of enzyme molecules on cytoplasmic 80 S ribosomes. In maize, it is reported that phytochrome activates 80 S ribosomes (Travies, Key & Ross, 1974). Therefore, it is possible that the enhanced activity of peroxidase could be due to the increased rate of enzyme synthesis on activated ribosomes in cytoplasm in the presence of Pfr.

The application of cycloheximide, a potent inhibitor of protein synthesis, however, gave different results, as compared to puromycin (Table III). At a concentration of

5 μg/ml, it differentially increased the peroxidase activity both in dark and far-red irradiated plants, whereas at a concentration of 20 μg/ml, the magnitude of enhancement was equal in both dark and far-red irradiated plants and was less than that obtained with low concentrations. These results suggest the possible existence of an inactivator of peroxidase with a rapid turn-over rate, perhaps similar to that reported for phenylalanine ammonia lyase (Engelsma, 1970) and invertase (Pressy, 1967). It is possible that at low concentration, cycloheximide inhibits synthesis of inactivator but not of peroxidase, thereby resulting in an enhanced activity of peroxidase. At higher concentration, cycloheximide may be inhibiting the peroxidase synthesis also, thereby reducing the magnitude of enhancement. Creasy, Zucker & Wong (1974) have also shown an identical response on comparing cycloheximide-mediated inhibition of phenylalanine ammonia lyase activity at different concentrations. They found that the inactiviation was much more sensitive to cycloheximide than phenylalanine ammonia lyase formation, which is consistent with our results.

TABLE II

Effects of inhibitors of protein and RNA synthesis on phytochrome-mediated increase in peroxidase activity in apical leaves of maize

Treatment	Peroxidase activity (units/mg protein)	Relative enzyme activity (%)
1. 7 days d (H_2O)	33.0	100
2. 6 days d (H_2O)+24 h d (Act-D 8 μg/ml)	31.0	94
3. 6 days d (H_2O)+24 h d (PUR 100 μg/ml)	34.0	103
4. 6 days d (H_2O)+ 24 h d (CAP 50 μg/ml)	36.0	109
5. 6 days d (H_2O)+24 h d (CAP 250 μg/ml)	36.0	109
6. 6 days d (H_2O)+24 h FR (H_2O)	61.0	183
7. 6 days d (H_2O)*24 h FR (Act-D 8 μg/ml)	57.0	170
8. 6 days d (H_2O)+24 h FR (PUR 100 μg/ml)	32.0	97
9. 6 days d (H_2O)+24 h FR (CAP 50 μg/ml)	59.5	179
10. 6 days d (H_2O)+24 h FR (CAP 250 μg/ml)	59.0	177

Act-D, Actinomycin-D; *PUR*, Puromycin; *CAP*, Chloramphenicol; *FR*, Far-red light; *D*, Dark.

TABLE III

Effect of cycloheximide on phytochrome-mediated increase in peroxidase activity in apical leaves of maize

Treatments	Peroxidase activity (units/mg protein)	Relative enzyme activity (%)
1. 7 days d (H_2O)	33.0	100
2. 6 days d (H_2O)+24 h d (CHI 5 μg/ml)	56.2	170
3. 6 days d (H_2O)+24 h d (CHI 20 μg/ml)	44.6	135
4. 6 days d (H_2O)+24 h FR (H_2O)	61.0	183
5. 6 days d (H_2O)+24 h FR (CHI 5 μg/ml)	67.8	205
6. 6 days d (H_2O)+24 h FR (CHI 20 μg/ml)	44.0	133

CHI, Cycloheximide; *FR*, Far-red light; *D*, dark.

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