Action and Interaction of Red and Far-Red Radiation on Lipoxidase Metabolism of Squash Seedlings

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Summary. Lipoxidase, in the cotyledons of squash (Cucurbita moschata) seedlings grown in the dark, reached its peak activity on the fifth day and then declined to its lowest activity on the eighth day. Under continuous irradiation, the rate of enzyme disappearance was accelerated by red (655 mµ) and was retarded by far-red (735 mµ) radiation. Acceleration of enzyme disappearance caused by red light was reversed repeatedly by far-red light in seedlings that received an initial exposure to red radiation. These responses were independent of the duration of irradiation at each of the alternating wavebands. No change was observed when the white light was administered either 24 hours before or 24 hours after the red, far-red treatment.

The lipoxidase system of the seedlings given an initial exposure to far-red radiation also responded reversibly to alternating far-red, red extended exposures, but it failed to respond reversibly when short exposures were employed. Similarly, no change occurred in these seedlings when either pre- or post-treatment with the white light was applied.

These results demonstrate that the capacity of lipoxidase to act reversibly depends primarily on the duration of exposure and on the kind of light (red or far-red) to which the seedlings were exposed initially. In spite of these variations, lipoxidase metabolism can be considered an additional biochemical manifestation of red, far-red reaction that operates in the photomorphogenesis of plants.

Previous studies on the effect of light on lipoxidase metabolism in the cotyledons of squash seedlings indicated that enzyme activity decays in the dark and the rate of enzyme decay increases as both the light intensity and exposure times increased. This light-dependent disappearance of enzyme response was correlated with the known characteristic features of the reaction presumed to be brought about by the high energy reaction (6). During the course of this study, phytochrome was detected in the cotyledons where lipoxidase was also heavily localized. The time-course study indicated that both lipoxidase and phytochrome are either absent or dormant in the dry seeds; both are activated at about 48 hours from the start of hydration, reach their maximum activities at about the fourth or fifth day and then decline (6). The similarities in their patterns of activation and disappearance, their heavy concentrations in the cotyledons and the sensitivity of lipoxidase to light, suggested a causal relationship between these 2 physiological systems.

The present study was, therefore, designed to observe if red or far-red-induced responses in lipoxidase activity could be reversed by a subsequent exposure of the seedlings to far-red or red radiation in a repetitive sequence. The results of these investigations are reported here.

Methods and Materials

Squash seeds (Cucurbita moschata var. Early Prolific Straight) were shelled by hand, soaked in water for about 5 hours and planted in plastic boxes containing water-saturated vermiculite covered with about 1 cm of washed sand. The boxes were kept in the dark at 25°C at about 80% relative humidity. After 4 days, these dark-grown seedlings were given alternating red, far-red light treatments and then returned to the dark room. This dark room was equipped with a safe light (2), but the seedlings were always protected from direct exposure to this light. The first sample of 20 cotyledons was collected on the fifth day for the determination of lipoxidase activity. Sampling was repeated after every 24 hours till the end of the experimental

1 Work performed under the auspices of the United States Atomic Energy Commission.
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period, i.e., the eighth day from the start of hydration.

For the red, far-red irradiation, monochromators fitted with interference and infrared-reflecting filters were used (10). The white light was administered from a luminaire of a mixed fluorescent tube-tungsten lamp at a wattage ratio of 5 to 1.

The enzyme was prepared from the cotyledons according to the procedure described earlier (6). Briefly, this procedure was as follows: Cotyledons were ground in sand and the pigments were repeatedly extracted with diethyl ether. The ether-free residue was extracted in a known volume of cold sodium phosphate buffer. The suspension was centrifuged and the lipoxidase activity of the supernatant liquid was determined spectrophotometrically (4, 5, 6).

Previously, it was found (6) that lipoxidase activity in the cotyledons of dark-grown squash seedlings commenced at about 48 hours, reached a peak at about the fifth day and then declined exponentially to its lowest level on the eighth day. The method for the statistical representation of data employed is the same as was used in previous reports (5, 6); i.e., the decline in zero-order activity rates, from the fifth through the eighth day, is a decay constant, and the change in the magnitude of this rate constant represents the effect of the light to which the seedlings were exposed.

![Graph showing the effect of red and far-red irradiation on lipoxidase activity in cotyledons.](image)

**Fig. 1.** Effect of red (655 m\(\mu\)) and far-red (735 m\(\mu\)) radiations on lipoxidase activity in cotyledons. Four-day etiolated seedlings were exposed to equal incident flux, 40 \(\mu\)W/cm\(^2\), of red and far-red light for the indicated times. Following irradiation, seedlings were removed to darkness and the zero-order activity rates (per cotyledon per minute) were determined each day from the fifth through the eighth day. Each datum is a decay constant for the zero-order activity rates and each curve shows change in decay constants caused by the geometric increase in exposure time. Decay constants for the dark controls ranged between -0.6 to -0.7 ± from 0.06 to 0.15. Standard errors in decay constants for red and far-red varied between 10 to 40% of the indicated values.

![Graph showing repetitive reversibility of lipoxidase metabolism in cotyledons of squash seedlings.](image)

**Fig. 2.** Repetitive reversibility of lipoxidase metabolism in the cotyledons of squash seedlings. One group consisting of 6 batches of 4-day old etiolated seedlings was exposed to red and the other similar group to far-red light. After 90 minutes of irradiation one sample from each group was transferred to darkness and the remaining 5 samples were mutually exchanged. After every 90 minutes thereafter removal and mutual exchange was repeated until all the samples had been removed to darkness. Decay constants for enzyme activity were determined as described for figure 1. Left: initial exposure to red (R) followed by far-red, red light cycles. Right: initial exposure to far-red (F) followed by red, far-red light cycles. Top: radiation flux for red and far-red was 40 \(\mu\)W/cm\(^2\); Bottom: radiant density of far-red was increased from 40 to 80 \(\mu\)W/cm\(^2\), i.e., red to far-red ratio was 1:2.

**Results**

*Continuous Red, Far-Red Irradiation.* The decay curves for lipoxidase activity in the cotyledons of squash seedlings exposed to red and far-red light are shown in figure 1. It is shown that while the decay process increased as the exposure time increased, the enzyme depleted faster in the red than it did in the far-red irradiated seedlings. The rate of enzyme decay caused by far-red remained low for the first 90 minutes but it approached the levels of the dark controls when the exposure time was increased; the rates of enzyme disappearance in red light matched the decay constants of the unirradiated samples during the first 45 minutes but increased thereafter (fig 1).

*Alternating Red, Far-Red Irradiation.* Responses of the seedlings irradiated for 90 minutes
at each alternating waveband are shown in the top part of figure 2. It shows that far-red retarded enzyme depletion; the effect of far-red was counteracted by red light and repeated reversals occurred regardless of whether the irradiation sequence began with red (left) or with far-red light (right). Whether this reversal pattern remained unchanged or was augmented by increased irradiance of far-red relative to red radiation was determined next. Changing the ratio of red to far-red light from 1:1 to 1:2 (fig 2, bottom) also caused no significant change in the capacity of the system to respond reversibly: The main difference, however, was that the seedlings given an initial exposure to far-red light (bottom right) maintained an elevated net metabolic level and the differences between the red and the far-red responses were less than differences induced in the corresponding samples irradiated first with red light (bottom left).

Since repetitive reversals occurred equally well when the ratio of red to far-red irradiance was either 1:1 or 1:2, the remaining experiments, employing shorter exposures, were conducted with an irradiance ratio of 1:2.

Seedlings given exposures shorter than 90 minutes at each alternating waveband showed essentially similar responses. The red-accelerated and far-red-retarded responses of enzyme disappearance continued to be mutually reversed only when the irradiation sequence began with the red light. In the complementary experiments where the irradiation sequence began with the far-red light, no repeated reversal by the red light was observed (fig 3).

Pre- and Post-Treatment with White Light. In order to observe if the white light has any effect on these reversible responses, the seedlings were exposed to the white light either before or after the red, far-red treatment. The results of these experiments are shown in figure 4. The top part of this figure shows the decay constants for seedlings that received 3 hours of white light, 24 hours after the red, far-red (left), or after far-red, red (right) light cycles. The lower part of this figure represents samples that were given a brief exposure to the white light 24 hours prior to exposures to antagonistic wavebands. Qualitatively, the results obtained with both pre- and post-treatment agree with each other; They also agree with the responses of samples that were handled the same way, but without the pre- or post-treatment to white light (fig 3, bottom).

Discussion

The sensitivity of lipoxidase to prolonged light exposures suggested a strong correlation with the known photoresponses presumed to be controlled by the high energy reaction (6). Lipoxidase also

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Fig. 4. Top: same procedure as for figure 3 (bottom) except that the samples were given 3 hours of white light (2000 ft-c) after 24 hours of red, far-red treatment, i.e., on the fifth day. As usual, the sampling was repeated from the fifth through the eighth day. Bottom: the 3-day etiolated seedlings were given 1 minute of white light (2000 ft-c), returned to darkness to complete 4 days of growth. They were then treated the same way as described for figure 3 (bottom).
responded to the red, far-red (low energy) reversible reaction, but the duration of light exposures for which the reversals are demonstrated (e.g., fig 2) far exceeded the limits of irradiation normally employed for the induction of photomorphogenic responses mediated by phytochrome. Even though phytochrome was present in the cotyledons, it is not known whether these responses were mediated indirectly by the photoreceptor or whether they were affected directly by the red and far-red radiations.

Sale and Vince (1) reported that 4 hours of red light-induced leaf-expansion could be partly prevented by 4 hours of subsequent far-red radiation. They did not study the effect of far-red followed by red radiation. Neither did they demonstrate the red, far-red repetitive effect. The observations of Sale and Vince (1) together with the observations on lipoxidase reported here, indicate that the phenomenon of photoreversibility, as applied to the low energy reaction, is not restricted to short exposures only. In fact, lipoxidase metabolism responded to reversible reaction much better when the exposure times were longer than it did in response to short exposures (fig 2 vs. fig 3).

This study also demonstrates that red and far-red radiations do not always counteract the effect of each other. Although reversibility is determined by the wavelength to which the seedlings are subjected last, the duration as well as the quality of initial light (red or far-red) exposure is equally important in determining the capacity of the system to respond reversibly. For example, the effect of red light was repeatedly reversed by far-red in the seedlings exposed initially to red light, but the effect of far-red was not reversed by red light when the initial exposure was to far-red and when the exposure times were short. This suggests that the optimal ratio of red to far-red light may be different for the seedlings exposed first to red than it is for the seedlings in which the irradiation sequence began with far-red light. These ratios may be critical especially when short exposure times are employed.

Similar differential responses induced by the initial exposure to red or to far-red radiation have been reported for flower induction (8), and for other red, far-red dependent metabolic responses (3, 7, 9). In spite of the differences between known photomorphogenic responses and the responses of lipoxidase observed here, this study presents additional evidence of red, far-red (low energy) reaction that operates in plants.

Literature Cited