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Rapid Phytochrome-mediated Changes in Adenosine 5'-Triphosphate Content of Etiolated Bean Buds[1]

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ABSTRACT

This study was designed to determine the effects of red and far red irradiation on ATP metabolism in etiolated bean buds (*Phaseolus vulgaris* L. var. Red Kidney). Compared to dark controls, red irradiated buds show an initial decline in ATP content at 15 seconds following a 5-minute irradiation. ATP content then rapidly rises to a peak at 1 minute, and then slowly returns to the baseline. The 1-minute promotion of ATP content is red/far red reversible. Acetylcholine does not appear to mimic red light in this system; it causes a marked decrease in ATP content.

Various theories have been invoked to explain the action of phytochrome (4, 13, 20, 21). One factor that seems to underlie all of these mechanisms is a dependence on a supply of energy, presumably in the form of ATP. Thus, gene activation would demand ATP in the amino acid activation and polymerization reactions, while changes in membrane permeability and electrical activity would probably depend on the activity of ATP-driven ion pumps (24).

Many rapid energy-dependent phytochrome actions seem to involve events at cellular membranes. Phytochrome appears to control the adhesiveness of root tips to glass surfaces (30, 31). Exogenously supplied ATP was required for the release of root tips, and endogenous ATP may be required for adhesion (32). Jaffe (15) correlated these changes with light-induced alterations in root tip electrical potential. Newman and Briggs (22) demonstrated rapid phytochrome-mediated changes in potential in intact *Avena* coleoptiles. The changes were most prominent in the upper part of the coleoptile, an area demonstrated by Briggs and Siegelman (5) to be particularly rich in phytochrome. Tanada (31) had shown changes in root tip adhesiveness within 30 sec following irradiation, and Newman and Briggs (22) could detect electrical potential changes within 15 sec following the onset of illumination. The nyctinastic leaflet movements of *Albizzia julibrissin* are dependent on ion fluxes, accompanied by osmotic water movement, in the pulvinules (24, 25). The flux of ions might be a consequence of an ATP-dependent, membrane-localized pump. Jaffe and Thoma (16) recently demonstrated an increase (compared to dark roots) in acetate uptake by etiolated mung bean root tips during a 4-min red irradiation, and there is evidence for a red light-induced H^+ efflux (33).

Other rapid responses do not necessarily involve action on membranes, though the importance of high energy compounds is evident. Yunghans and Jaffe (33) found that red irradiation of etiolated mung bean root tips caused rapid changes in the level of inorganic phosphate and the rate of O_2 uptake. Manabe and Furuya (19) reported that within 2 min of a red irradiation, there was a large increase in the content of NADPH in a particulate fraction obtained from etiolated bean hypocotyls. The NADPH level rose only slightly following far red irradiation.

It is possible, therefore, that changes in cellular ATP content might be a consequence of phytochrome action. Phytochrome might act directly on reactions consuming or generating ATP or both, or changes in ATP level may be a rather nonspecific consequence of alterations in over-all cellular activity. Sisler and Klein (27) reported that red and far red light do not alter ATP levels in etiolated bean leaves or hooks or in *Avena* mesocotyl or coleoptile segments. However, the first time point analyzed was 1 hr following irradiation. Yunghans and Jaffe (33) report that red light irradiation of 6-day-old etiolated mung bean root tips caused the concentration of ATP to fall by 12-fold. The ATP level approximated dark control values following far red irradiation. The animal neurohumor acetylcholine mimicked the effects of red light, so Yunghans and Jaffe (33) suggested that red irradiation induced the extremely rapid synthesis of ACh,[4] which then acted at target sites, perhaps causing rapid changes in membrane permeability. Although other reports suggest phytochrome control of ACh levels (12), the effect is not universal (17). No effects of red irradiation on ACh levels were seen in etiolated pea buds or in *Albizzia* pulvinules (23). The red light-induced acetate uptake in mung bean roots was blocked by the ACh antagonist *d*-tubocurarine and was enhanced by the acetylcholinesterase inhibitor AMO-1618 (16).

We report here rapid changes in ATP levels in etiolated bean buds. Bean buds are rich in phytochrome, and phytochrome is known to induce many morphogenetic changes in this tissue (8).

MATERIALS AND METHODS

Plant Material. Seeds of bean (*Phaseolus vulgaris* L. var. Red Kidney) were obtained from P. L. Rohrer and Bro., Smoketown, Pa. Seeds were surface-sterilized with 2.5% sodium hypochlorite, rinsed in distilled water, and planted on two layers of moistened Kimpak 6233 (Kimberly-Clark). The plants were grown in total darkness in an incubator at 27 C for

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[4] Abbreviation: ACh: acetylcholine chloride.

6 days. Apical buds were removed at the first node and randomized. Sets of buds (four per treatment) were weighed and then placed on nylon netting in Petri dishes containing deionized water.

Irradiation Procedure. All manipulations were carried out under dim green safelights. Green incandescent bulbs filtered through two layers of dark green and one layer of amber celluloid and fluorescent bulbs filtered through amber and blue Plexiglas (Rohm and Haas) (9) were used. Red and far red irradiations were carried out in separate light-tight chambers, essentially similar in design and light output to those described by Fosket and Briggs (7). Red light was provided by five red fluorescent tubes (Westinghouse F20T12/R) filtered through 3 mm of red Plexiglas (No. 2423, Rohm and Haas). The far red source consisted of nine 150-w incandescent bulbs filtered through 3 mm of FRF-700 Plexiglas (Westlake Plastics) and 5 cm of water. Irradiations were of 5-min duration. These sources supplied light dosages sufficient to saturate a test bioassay, the photoreversible control of Grand Rapids lettuce seed germination (3).

Extraction and Assay of ATP. All glassware was acid-cleaned. Dark periods of various durations were interposed between irradiation and extraction. Near the end of the dark period, the nylon netting containing the buds was removed, blotted dry on filter paper, and, at the specified time, plunged into liquid N_2 for 1 min. The frozen buds were then transferred to a glass homogenizer tube with 0.6 ml of arsenate buffer, 0.05 M, pH 7.4, and boiled for 1 min. The tissue was then homogenized with a Teflon pestle, and the extract was chilled on ice. The homogenate was centrifuged at 3000*g* for 5 min, and the supernatant was stored on ice for assay within 2 hr.

ATP was assayed by means of the luciferin-luciferase assay (1, 6, 27). Frozen and desiccated firefly lantern extract (Sigma Chemical Co.) was dissolved in deionized water and diluted in arsenate buffer made to 20 mM in $MgCl_2$. The enzyme was stored on ice for 48 hr before use to reduce background (26), and filtered through Whatman No. 1 paper just prior to assay.

Assays were conducted in small vials fixed inside of scintillation vials. Determinations were made on a Nuclear Chicago Model 6868 liquid scintillation counter set to program 11. A 0.1-ml portion of firefly extract was placed in the inner vial. The test sample (0.2 ml) of bean bud extract was then forcibly added with a syringe, the scintillation vial capped, and the assembly lowered into the counter. Consistent, rapid (5 sec) procedure was followed. Extracts were diluted to yield activity in the range of 20,000 to 100,000 cpm. Each extract was tested in four replicate trials. A standard curve of activity was determined each day using known concentrations of ATP in arsenate-$MgCl_2$ buffer. Though enzyme activity varied from day to day, the response was linear at least between 7×10^{-9} M and 10^{-7} M ATP, the region used for experimental trials. Data (in cpm/gm tissue) were expressed as percentage of dark controls (normalized to 100%) run at the same time.

Incubations on Acetylcholine. ACh (Sigma Chemical Co.) was made up in deionized water. Buds were blotted dry and incubated on test solutions or water for 6 min (to be equivalent to 5 min of light treatment plus 1 min of dark incubation). ATP was then extracted and assayed. ACh at the tested concentrations had no effect on the firefly extract light emission elicited by standard ATP solutions.

RESULTS

The time course for changes in ATP levels during darkness following red irradiation is presented in Figure 1. At 15 sec following irradiation, the ATP level has declined to 78% of the dark control value. The ATP level then rises to 192% of dark control at 1 min, and then slowly returns to a level near the baseline. A one-way analysis of variance showed that time was a significant variable affecting ATP levels in red irradiated etiolated bean buds. Dark control samples showed essentially no change in ATP content over the 30-min time span.

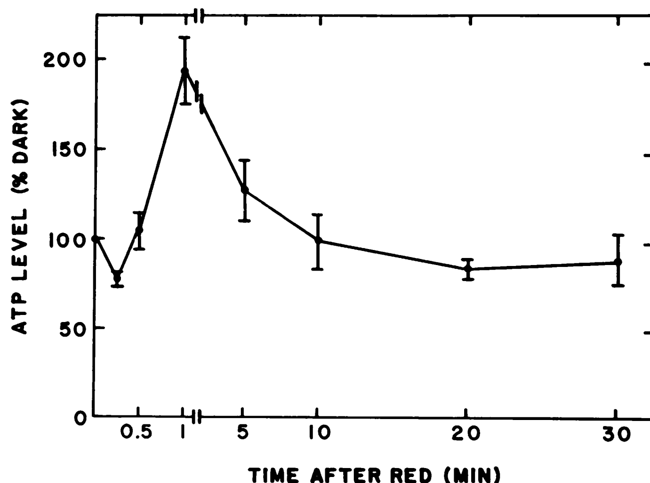

FIG. 1. Effects of red irradiation (5 min) on ATP levels. Times indicated are the lengths of postirradiation dark periods. Dark control values were normalized to 100%. Values are mean ± SE of at least five replicates tested on different days.

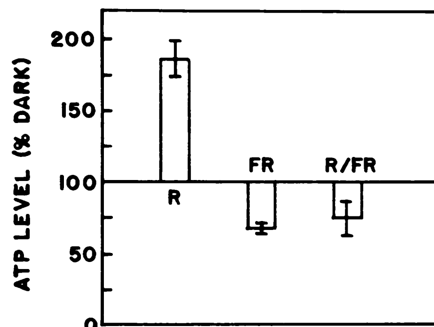

FIG. 2. Effects of various light treatments (5 min) on ATP levels after 1-min dark period following irradiation. Dark control values were normalized to 100%. Values are mean ± SE of at least five replicates tested on different days.

Since the most dramatic response was observed 1 min following red irradiation, this point was chosen for a determination of red/far red photoreversibility. The results are shown in Figure 2; the red/far red sample was transferred from one light source to the other within 5 sec. The sharp increase in red-induced ATP content repeats the finding of Figure 1. Far red caused a decrease in ATP content below the level of the dark controls, and the red/far red treatment produced a similar effect. A one-way analysis of variance showed the light treatment to be a significant variable affecting ATP levels below the 0.005 level of significance. A Newman-Keuls analysis showed red treatment to be significantly different from far red, red/far red, and dark treatments, while far red, red/far red, and dark treatments were not significantly different from one another.

Because of the rapidity of the red-induced changes in ATP concentration, this conventional sort of photoreversibility experiment may be questioned. Since the far red irradiation takes 5 min, the red/far red value (Fig. 2) can be compared with the 5-min point on the red-induced time course (Fig. 1). By a Stu-

Table I. *Effect of Acetylcholine on ATP Levels in Etiolated Bean Buds*

Buds were incubated for 6 min on test solutions or water before ATP extraction. There were at least five replicates of each treatment.

Acetylcholine Concn	ATP Content
M	*% water control*[1]
10^{-3}	31 ± 2
10^{-5}	37 ± 6
10^{-7}	22 ± 3
10^{-9}	30 ± 6
10^{-11}	29 ± 6

[1] Mean ± SE.

dent's *t*-test, the former value (74% of dark control) is significantly different from the latter (127%) at the 97.5% confidence level.

ACh was tested in the concentration range 10^{-3} to 10^{-11} M. As shown in Table I, ACh induces a decline in ATP content to about 20 to 40% of water-incubated controls. Clearly, the 6-min incubation period is sufficient for the compound to exert effects on the tissue, but there is no indication of a dosage response. The potentiation of a response at 10^{-11} M is striking.

DISCUSSION

ATP levels in etiolated bean buds are influenced rapidly by brief red irradiation. Changes are observed 15 sec following a 5-min irradiation, and it may be possible to detect changes even more rapidly by using a more intense light source to deliver the same total energy, as Newman and Briggs (22) did in their electric potential determination. The time course of Figure 1 can perhaps suggest a reason for Sisler and Klein's (27) failure to detect a change in ATP content in the same experimental system. Their first time point was at 1 hr postirradiation, and we showed an apparent return to dark control values by 30 min.

Figure 2 shows that the effects of red and far red light alone are significantly different. Concerning the red/far red schedule, it is possible that in the time it takes to deliver the far red irradiation following the red, changes induced by red could become manifest. The same problem is evident in Manabe and Furuya's (19) report on changes in NADPH levels. Clearly, some way of saturating pigment phototransformation almost instantaneously would be desirable. Such a system would allow the repeated red/far red treatments necessary to define a classical phytochrome response. But, taken together, the data of Figure 2 and the comparison with the 5-min point of Figure 1 do suggest that far red can at least partially reverse the effect of red. Thus, the effect reported here may be described as phytochrome-mediated.

The changes in ATP content shown in Figure 1 are the summation of ATP production and utilization in many processes, such as anabolic and catabolic reactions, the operation of transport systems, or the maintenance of electrochemical gradients across membranes. Changes in crucial coenzymes such as ATP may have wide ranging effects on cellular metabolism. For example, Manabe and Furuya (19) have shown rapid, phytochrome-dependent increases in NADPH levels, which may result from the activation of glucose-6-P dehydrogenase. Most other direct assays of key metabolic enzymes have been made after long irradiations or long postirradiation dark periods (8, 28). The observed phytochrome-mediated changes in O_2 uptake suggest an effect of light on the activity of the mitochondrial oxidative phosphorylation system or of Krebs' cycle or Embden-Meyerhof pathway enzymes. At present, we do not know which system generates the ATP we are measuring. Since there is a depletion of polyribosomes under anaerobic conditions (18), the ATP level could rapidly influence the protein synthesizing machinery.

If phytochrome is localized in the cell membrane (or exerts its action there), then alterations in chromoprotein conformation (14) may affect the structure and hence activity of neighboring membrane-bound proteins. Formation of Pfr may alter the activity of ATPases, although Yunghans and Jaffe (33) could detect no red light-induced changes in root tip ATPases. Pfr may modulate the activity of ATP-dependent ion pump systems (24).

Previous reports of phytochrome-mediated phenomena have not indicated the complex time course shown here. Interestingly, the time course for red-induced changes in coleoptile electrical potential showed a peak within 2 min of irradiation, followed by a slow decline to the baseline region by about 10 min (22). Though the irradiation periods used in this study and those employed by Newman and Briggs (22) were different, there is some similarity in the decay of the response after the peak.

Yunghans and Jaffe (33) reported a decline in root tip ATP levels of about 12-fold following a 4-min red irradiation. They suggest that the elapsed time between turning off the light and grinding the tissue was very short. Nonetheless, we could detect only a slight decline (about 20%) in bean buds, followed by a large increase. Jaffe and Thoma (16) suggest that the roots' ATP content declines as ATP is used to drive ion uptake. Since changes in ATP content reflect changes in rates of synthesis and degradation, differences between the time courses observed in buds and roots may reflect differences in the light stimulation or retardation or both of various processes in the two tissues. An elaboration of the time course of light-induced ATP changes in roots might resolve this difference. The rapid adhesion-release phenomenon has only been reported in root tips (30, 31), but electrical changes in the same direction (red caused the apical region to become relatively electropositive) were shown in roots (15) and shoots (22).

Based on the observation that ACh mimics red light in several of the root tip responses, Yunghans and Jaffe (33) concluded that a primary response to the formation of Pfr is an extremely rapid synthesis of ACh. They further hypothesized that ACh may stimulate ATP-dependent ion transport systems in the membrane. Other experiments suggest an ACh-mediated acetate uptake as a consequence of red irradiation (16). Our results do not seem to support Yunghans and Jaffe's theory (33), because nowhere on the curve for red-induced ATP changes do ATP levels decline as dramatically as when ACh is exogenously supplied. The kinetics of ACh uptake by bean buds is unknown, so a direct comparison of the 6-min ACh treatment to the time course (Fig. 1) should not be made. The lack of a dosage response to ACh also suggests that ACh is not regulating phytochrome-mediated ATP changes in bean buds, but this finding does not directly bear on Yunghans and Jaffe's hypothesis. An analysis of light-induced changes in endogenous ACh is necessary, although experiments designed to detect a red light/ACh synergism would be useful. Our results are consistent with the suggestion that red light may act through ACh in plant organs adapted to an aqueous environment, but not in those adapted to an air environment (17). This difference might result from differences in the structure of shoot and root cell membranes. There may well be different controls on various aspects of cellular ATP metabolism.

There is now evidence for the involvement of cyclic 3',5'-adenosine monophosphate in certain plant morphogenetic responses (10, 11). Since ATP is the precursor for this regulator and since, in animal systems, adenyl cyclase is membrane-bound (29), we are currently investigating the involvement of the cyclic AMP system in our response. An attractive model for further investigation is the frog visual photoreceptor membrane, in which light may induce changes in membrane ion flux and hence changes in electrical potential by affecting the activity of adenyl cyclase (2).

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