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# Simultaneous Phytochrome-controlled Promotion and Inhibition of Arginine Decarboxylase Activity in Buds and Epicotyls of Etiolated Peas[1]

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ABSTRACT

**The specific activity of arginine decarboxylase (ADC; L-arginine carboxylase; EC 4.1.1.19) rises steadily over an 8 hour experimental period in the growing buds and subapical epicotyl internodes of 6-day-old totally etiolated pea seedlings. Treatment with red light (R) completely annuls this rise in epicotyls but increases it in buds, thus paralleling the opposite effects of R on the growth of these two organs. Far red light (FR) reverses both effects of R on ADC and is, in turn, reversed by R, indicating phytochrome control. Effects in both organs are clearly seen within 2 hours. By 6 hours after R, the post-irradiation rise in ADC specific activity in buds is 3 times greater than that of the dark controls. Over the same period, ADC specific activity in epicotyls is inhibited by 56% relative to dark controls, reflecting zero net change after R and a continued rise in the dark. Cycloheximide inhibits the rise in ADC activity in both rapidly growing organs (epicotyls in dark and buds after R) but is without effect in both slower growing organs. Actinomycin D inhibits only in dark grown epicotyls, whereas chloramphenicol produces no inhibition in any system tested.**

**ADC is the first enzyme to show a two-way, organ-specific response to phytochrome conversion from Pr to Pfr. This finding is discussed in relation to the growing evidence that polyamines formed from arginine may be important growth regulators in plants, as well as in microbial and animal cells.**

Since the first report of phytochrome control of enzyme activity in 1960 (11), there has been great interest in the possibility that such regulation may be causally connected to photomorphogenetic events (18). Although the exact mechanism through which phytochrome regulates enzyme activity remains controversial, effects on both transcription and translation have been reported in diverse systems (4, 12, 15, 18).

In recent years, the ubiquitous polyamines putrescine, cadaverine, spermidine, and spermine have been shown to be capable of regulating important steps in DNA replication, transcription, translation, the cell cycle, and certain morphogenetic events (2, 5, 17, 19). Although the bulk of this work has been carried out with microbial and animal systems, some recent work has implicated polyamines as regulators of plant developmental processes (1, 7, 9, 13, 19). In particular, ADC,[3] a key enzyme in the polyamine biosynthetic pathway in plants, greatly increases in activity during growth in several plant systems (7, 13, 21). Since phytochrome and polyamines act by regulating similar aspects of cell growth, a question arises as to their possible functional relationship. Thus far, there has been no report connecting phytochrome status with the activity of biosynthetic enzymes of polyamines (15, 18). Here, we present evidence for a two-way, organ-specific phytochrome regulation of ADC activity in buds and epicotyls of etiolated pea seedlings. Since the Pfr-induced changes in ADC activity parallel the opposite Pfr-induced growth changes in these organs, the possibility of a causal connection between polyamine level and growth is strengthened.

## MATERIALS AND METHODS

**Plant Material.** Seeds of *Pisum sativum* L. var. Alaska were imbibed for 10 h in tap water in the dark and sown in prewashed vermiculite. After 6 days in the dark at 27 C and about 70% RH, seedlings were selected for uniformity of third internode length (30–50 mm) and sharply recurved apical hooks.

**Light Sources and Irradiation.** The R source (600–900 nm) consisted of four 15-w red fluorescent tubes (Sylvania) wrapped with two layers of Dupont red cellophane. Energy at the seedling level was 2.0 kiloergs $cm^{-2}$ $s^{-1}$. The FR source (710–760 nm) consisted of five 300-w internal reflector incandescent flood lamps filtered through 16 cm water and a Westlake FR Plexiglas FRF 700 filter, emitting 19 kiloergs $cm^{-2}$ $s^{-1}$ energy at the seedling level. Both R and FR irradiations lasted for 5 min. After light treatment, the seedlings were kept in the dark for different time periods until harvest and assay. In the experiments involving photoreversibility, R or FR irradiation was followed immediately by FR or R, respectively.

**Enzyme Extraction.** For preparation of the crude enzyme extract, eight buds or eight subapical 5-mm epicotyl sections from the third internode were harvested under a photomorphogenically inactive green safelight (8) and ground in chilled mortar with 1 ml 100 mM phosphate (pH 7.0). The homogenates were centrifuged at 30,900*g* for 15 min at 4 C; the supernatant fraction was used for enzyme assay and protein estimation.

**Enzyme Assay.** ADC activity was determined by measuring labeled $^{14}CO_2$ release from DL-[1-$^{14}$C]arginine according to a method modified from Fong *et al.* (6). The reaction mixture consisted of 100-μl aliquots of crude enzyme, 7 μl DL-[1-$^{14}$C]-arginine (54 mCi/mmol) (New England Nuclear) diluted with unlabeled L-arginine to give a final concentration of 9 mM, and 10 μl 5 mM pyridoxal phosphate. Each reaction was carried out in triplicate in 10- × 75-mm polystyrene culture tubes sealed with plastic caps. A 7-mm diameter filter paper disc impregnated with 50 μl 2 N KOH and transfixed with a 22-gauge syringe needle through the cap was used to trap the $^{14}CO_2$ liberated. After incubation at 37 C for 30 min, the reaction was stopped and $CO_2$

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[3] Abbreviations: ADC, arginine decarboxylase; R, red light; FR, far red light; CHI, cycloheximide; CA, chloramphenicol; ActD, actinomycin D.

was released with 0.2 ml 10% trichloroacetic acid. The tubes were stoppered and incubated for an additional 30 min, after which the paper discs were removed, dried, and counted in minivials containing 2 ml Econofluor in an Ansitron scintillation counter. Enzyme activity is expressed as cpm/mg fresh weight or cpm/mg protein.

**Protein Determination.** Protein determinations in the crude enzyme were made by the method of Lowry *et al.* (10) using BSA as a standard.

**Application of Inhibitors.** Seedlings were sprayed with CHI (5 μg/ml), ActD (20 μg/ml), or CA (250 μg/ml) 45 min prior to the brief irradiations. All inhibitor solutions contained 0.05% pluronic L101 (Wyandotte Chem. Corp.) to aid penetration. Buffer solutions with pluronic L101 were used as controls.

**Replication of Experiments.** The data presented, unless otherwise stated, are from single experiments which are representative of a group of two to six experiments, each involving duplicate sets of experimental and control vessels.

## RESULTS AND DISCUSSION

**Effect of Phytochrome on ADC Activity in Epicotyls.** Seedlings irradiated with R for 5 min showed a progressive inhibition of the ADC activity of their epicotyls relative to dark controls; the effect was clear within 2 h and, after 6 h, ADC specific activity was inhibited 55%, compared to the dark control. During the period from 0 to 6 h, activity in the dark controls increased steadily; this increase was completely prevented for the full 6 h of the experiment by the brief R irradiation given at zero time (Fig. 1). A similar result had previously been reported for lipoxygenase in mustard cotyledons (14).

It is known that epicotyls elongate rapidly in the dark and that R partially inhibits this elongation in subapical segments of intact internodes of etiolated pea seedlings within 2 h (16, 22). The R-induced inhibition of the rise in ADC activity in subapical epicotyl tissue thus parallels closely the R-induced inhibition of epicotyl growth. Together with evidence that polyamines can promote macromolecular synthesis and growth in plants (1, 3, 9, 19), this prevention of the rise in ADC activity raises the possibility that the inhibition of epicotyl growth by R might be mediated through effects on polyamine biosynthesis.

The usual criterion for the involvement of phytochrome in a light-controlled response is that an effect of R should be reversed by subsequent FR irradiation. Figure 2 shows that 5 min R inhibited ADC specific activity by 44% and that this inhibition is completely reversed by 5 min FR irradiation given immediately after R. The FR reversal can in turn be reversed by subsequent 5-min irradiation with R. Thus, phytochrome involvement in control of ADC activity in pea epicotyls is clear.

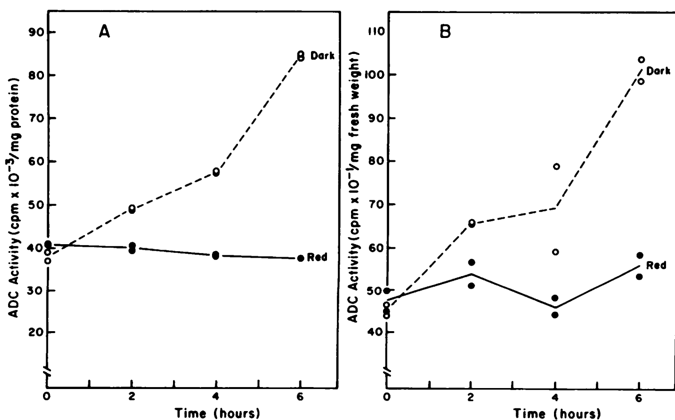


FIG. 1. The kinetics of change in ADC activity in epicotyls of etiolated pea seedlings. A, per mg protein; B, per mg fresh weight. (○- - -○), dark; (●——●), R. The duplicate data points of a single experiment are presented.

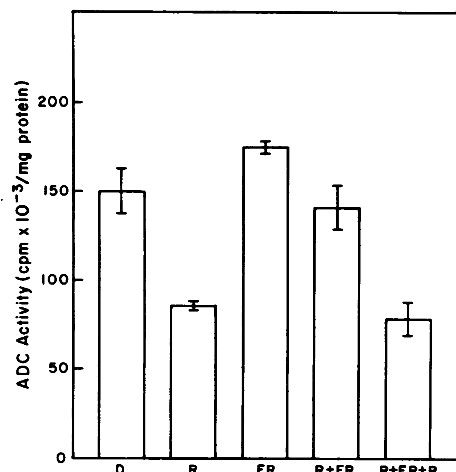


FIG. 2. FR reversibility of the R-induced inhibition of ADC activity in epicotyls of etiolated pea seedlings. Measurements from a single representative experiment made 4 h after light treatment. Bars indicate standard error of the mean. D, dark.

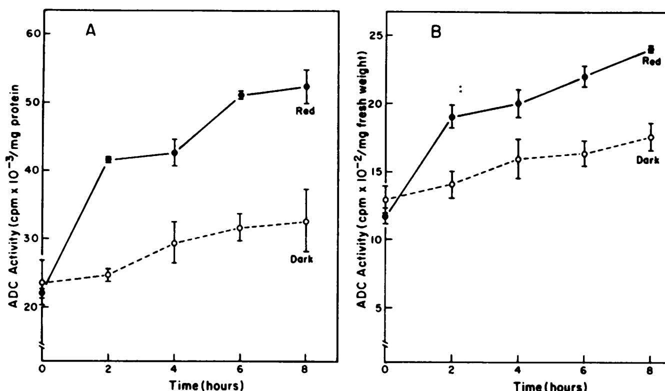


FIG. 3. The kinetics of change of ADC activity in buds of etiolated pea seedlings. A, per mg protein; B, per mg fresh weight. (○- - -○), dark; (●——●), R. Data points are average values for a single experiment involving duplicate flasks. Bars indicate standard error of the mean.

**Effect of Phytochrome on ADC Activity in Buds.** In contrast to the R-induced inhibition of the rise in ADC specific activity in epicotyls, the rise in ADC specific activity is promoted in buds irradiated with R (Fig. 3). In R-irradiated seedlings, ADC specific activity increased much more rapidly than in the dark control; after 8 h, it was 139% more than its initial value, whereas, in dark controls, the specific activity had increased by only 40% over the same period. The specific activities at 2 and 8 h after R treatment are, respectively, 68 and 60% over those of the dark controls. The figure shows that the major promotion of ADC activity occurred in the first 2 h after R irradiation; therefore, the difference between dark and R-irradiated material does not change further. Previous investigations have shown that brief exposure to R can promote the growth of pea buds within 3 h and that this promotion is reversed by FR (8). Thus, the promotion of ADC activity parallels the enhancement of bud growth by R.

In pea buds also, photoreversible R-FR control of ADC activity is clearly observed (Fig. 4). These data indicate that the promotion of ADC activity in buds is mediated by phytochrome, as is its inhibition in epicotyls.

Many reports indicate that, in animal systems, a large increase in the activity of a parallel polyamine-biosynthesizing enzyme, ornithine decarboxylase occurs during rapid growth (17, 20, 23).

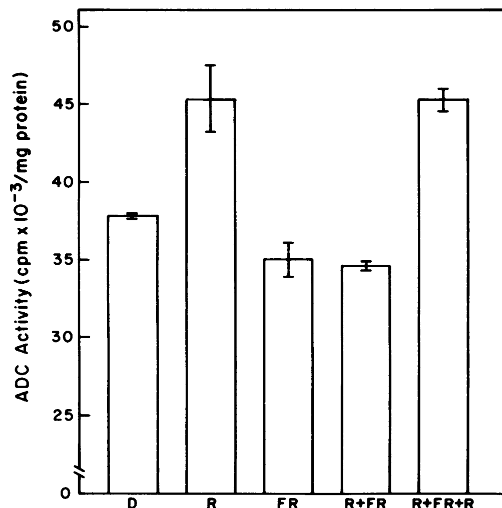


FIG. 4. FR reversibility of R-induced promotion of ADC activity in buds of etiolated pea seedlings. Measurements from a single representative experiment made 6 h after light treatment. Bars indicate standard error of the mean. D, dark.

Table I. *Effect of Some Inhibitors on Pfr-mediated Inhibition of ADC Activity in Epicotyls of Intact Etiolated Pea Seedlings*

All solutions contained 0.05% pluronic L101 to aid penetration. Measurement was made 6 h after R. Values are the average of two separate experiments done in duplicate.

| Light Treatment[a] | Solution | ADC Activity | Relative Activity | ADC Activity | Relative Activity |
|---|---|---|---|---|---|
| | | *cpm × $10^{-3}$/ mg protein* | % | *cpm × $10^{-2}$/ mg fresh wt* | % |
| D | Buffer | 60.51 ± 9.52 | 100[b] | 12.07 ± 2.36 | 100[b] |
| R | Buffer | 18.36 ± 1.71 | 31 | 4.79 ± 1.01 | 39 |
| D | Buffer + CHI | 39.73 ± 2.28 | 69 | 9.51 ± 1.68 | 80 |
| R | Buffer + CHI | 17.49 ± 1.41 | 30 | 4.83 ± 0.96 | 40 |
| D | Buffer + ActD | 39.61 ± 1.95 | 70 | 9.69 ± 1.31 | 84 |
| R | Buffer + ActD | 18.66 ± 0.72 | 34 | 4.69 ± 0.59 | 41 |
| D | Buffer + CA | 57.75 ± 9.65 | 95 | 12.35 ± 2.46 | 103 |
| R | Buffer + CA | 17.52 ± 2.55 | 29 | 4.49 ± 1.00 | 37 |

[a] D, dark.
[b] Buffer + pluronic in dark = 100%.

In plants, increasing evidence suggests that ADC activity as well as polyamine levels are elevated in growing tissues (13, 21). Thus the Pfr-induced promotion of ADC activity and growth in pea buds is logical and may indicate that the regulation of polyamine levels in buds is involved in phytochrome action.

**Effect of Inhibitors.** In darkness, both CHI (5 μg/ml) and ActD (20 μg/ml) inhibit the increase in ADC activity in epicotyls over a 6-h period to the same extent, about 30% (Table I). This finding indicates that the increased ADC activity is dependent upon both concomitant RNA and enzyme synthesis. These results are similar to those for lipoxygenase in mustard cotyledons (14) and are in accord with the data on CHI inhibition of increase in ADC activity in growing carrot and cucumber cells (13, 21). However, in R-irradiated epicotyls, where R induces an inhibition of rise in specific activity of ADC, treatments with CHI, ActD, and CA have no effect on ADC specific activity measured 6 h after R.

In buds, the interpretation of inhibitor effects is more complicated. In R-irradiated buds, the increase in ADC activity is inhibited only about 20% by CHI, slightly promoted by CA, and unaffected by ActD (Table II). These results suggest that the Pfr-

Table II. *Effect of Some Inhibitors on Pfr-mediated Stimulation of ADC Activity in Buds of Intact Etiolated Pea Seedlings*

All solutions contained 0.05% pluronic L101 to aid penetration. Measurements made 6 h after R. Values are the averages of three separate experiments, each done in duplicate.

| Light Treatment[a] | Solution | ADC Activity | Relative Activity | ADC Activity | Relative activity |
|---|---|---|---|---|---|
| | | *cpm × $10^{-3}$/ mg protein* | % | *cpm × $10^{-2}$/ mg fresh wt* | % |
| R | Buffer | 44.77 ± 4.74 | 100[b] | 21.17 ± 0.86 | 100[b] |
| D | Buffer | 31.09 ± 2.45 | 71 | 16.49 ± 0.79 | 78 |
| R | Buffer + CHI | 35.48 ± 5.41 | 77 | 15.45 ± 1.95 | 72 |
| D | Buffer + CHI | 27.90 ± 2.63 | 63 | 12.70 ± 0.65 | 60 |
| R | Buffer + ActD | 43.47 ± 4.60 | 97 | 18.97 ± 1.87 | 89 |
| D | Buffer + ActD | 34.48 ± 3.03 | 79 | 18.11 ± 1.35 | 85 |
| R | Buffer + CA | 49.62 ± 1.28 | 110 | 23.85 ± 2.39 | 112 |
| D | Buffer + CA | 36.31 ± 2.81 | 83 | 19.17 ± 1.44 | 90 |

[a] D, dark.
[b] Buffer + pluronic after R = 100%.

mediated increase in ADC activity may be due to enzyme synthesis on cytoplasmic 80 S ribosomes; the insensitivity of the process to CA and ActD probably excludes involvement of transcription and translation on ribosomes of organelles. Thus, it appears that the mechanism of increase in specific activity of ADC in R-irradiated buds is somehow different from the increase occurring in dark epicotyls. The unifying generalization is that, in both rapidly growing organs (dark epicotyls and R-irradiated buds), CHI inhibits the increase in ADC activity, whereas in both slower-growing systems (R-irradiated epicotyls and dark buds), it is without effect. Thus, translation may well be the process involved in the two-way, organ-specific control by phytochrome.

A recent comprehensive review (18) listed 52 enzymes whose activities are controlled by phytochrome. Arginine decarboxylase must now be added to that list, but with special emphasis because of its unique behavior. To our knowledge, it is the only enzyme showing simultaneous, organ-specific promotion and inhibition as a consequence of phytochrome transformation. It thus offers unique advantages in the study of the mechanism of phytochrome-controlled morphogenetic phenomena, especially since the direction of the changes in enzyme activity parallels the direction of the growth changes in each organ.

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