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Ion Fluxes and Phytochrome Protons in Mung Bean Hypocotyl Segments

II. FLUXES OF CHLORIDE, PROTONS, AND ORTHOPHOSPHATE IN APICAL AND SUBHOOK SEGMENTS[1]

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ABSTRACT

The active form of phytochrome (Pfr) decreased Cl^- uptake by subhypocotyl hook segments of *Phaseolus aureus* Roxb. and increased uptake by apical segments. Pfr had similar effects on Pi [^{32}Pi] uptake. Modulations of Pi [^{32}Pi] uptake were detectable 10 minutes following photoconversion. Pfr may modulate Pi influx across the plasmalemma. Pfr inhibited H^+ extrusion by subhook segments and enhanced extrusion by apical hook segments. No rapid effects on H^+ extrusion were found. Phytochrome may regulate a K^+-H^+ exchange process. The differential responses of the two regions of the hypocotyl are discussed with respect to Pfr-mediated changes in growth and development.

Several ion transport processes have been shown to be under phytochrome control (13). Schrempf *et al.* (19) demonstrated Cl^- fluxes linked to fluxes of K^+ in phytochrome-controlled leaflet movement in *Samanea*. Pfr has been found to inhibit total Pi uptake by mung bean hypocotyl segments cut immediately below the hook (21). In pea epicotyl segments, Pfr caused an accelerated deacidification of the surrounding medium, measured 4 h following the light treatment (11). Pike and Richardson (16) showed that Pfr enhanced H^+ extrusion by apical oat coleoptile segments in KCl solutions.

This paper investigates the phytochrome control of Cl^-, H^+ and Pi in mung bean apical and subhook segments and attempts to relate these fluxes with K^+ fluxes described previously (3). Possible mechanisms by which phytochrome can control ion transport processes and their implications for growth and development are discussed.

MATERIALS AND METHODS

Growth of Seedlings and Pretreatment of Segments. Three-day-old etiolated *Phaseolus aureus* Roxb. seedlings, grown as described previously (3), were used in these experiments. Samples containing 3 mm apical hypocotyl hook or 2.5 mm subhook segments (see Figs. 1 and 2) from 30 or 40 plants were pretreated 3 h in 0.5 mM $CaCl_2$ and $MgCl_2$, 25 C. All manipulations were carried out under a dim green safelight.

Light Sources. R[3] and F were obtained as described previously (3).

Measurement of Cl^- and Pi Uptake. For Cl^-, weighed samples were heated in 50 mM NaOH, filtered, and the filtrate collected. This procedure was repeated twice for each sample. Five ml of NaOH was used per g fresh weight of tissue. Four-ml aliquots of this extract, diluted as required, were mixed with 1 ml of HNO_3:CH_3COOH reagent (0.2 M HNO_3 + 20% [v/v] glacial CH_3COOH) and 4 drops of gelatin reagent (5 g gelatin + thymol blue + thymol blue reagent crystals, 60:1:1 [w/w], dissolved in 100 ml distilled H_2O). Cl^- was titrated amperomerically using an Aminco chloride titrator. Cl^- uptake was calculated from Cl^- content of tissue before and after an experiment. For measurement of Pi uptake, pretreated sections were immersed in 5 ml ^{32}Pi-labeled (1 μCi ml^{-1}) 50 mM K_2HPO_4/KH_2PO_4 buffer for 10 min in D or in D following R. Samples were then transferred to identical, unlabeled solutions at 0 C for various times in D. Weighed samples were then dried and counted for radioactivity in a mixture of 1 ml distilled H_2O + 10 ml NE260 liquid scintillation fluid (Nuclear Enterprises Ltd., Edinburgh, U.K.) using a Packard Tri-Carb liquid scintillation counter. Wash solutions were counted as 1-ml sample + 10 ml scintillation fluid.

Measurement of H^+ Extrusion. The pH change of the bathing medium containing excised hypocotyl segments was measured using an Activion M22DE semimicro pH electrode in connection with a Corning EEL model 109 digital pH meter. Pretreated segments were immersed for 30 min in 5 ml aerated 1 mM KCl + 0.5 mM $CaCl_2$, and adjusted to pH 6.5 with 1 mM KOH. Samples were then transferred to 5 ml of fresh identical aerated solutions (pH 6.5) and either kept in D or irradiated with R or R followed by F. The pH of the medium was measured at hourly intervals. Samples were weighed at the end of each experiment. pH readings were converted to H^+ and H^+ extrusion was expressed as μmol (g fresh weight)$^{-1}$.

Statistical Analysis. Results are expressed as means of four replicates ± SE and were tested for significance using Student's *t* test (4) and repeated at least once with qualitatively similar results. Significant differences are at least at the 5% level unless otherwise indicated.

RESULTS AND DISCUSSION

The effect of R on Cl^- uptake by apical and subhook segments is shown in Table I. R caused a decrease in total uptake by subhook segments and an increase in total uptake by apical hook

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[3] Abbreviations: R: red light; F: far red light; D: dark; R/F: red light followed by far red light.

Table I. *Total Uptake of Cl^- by Apical and Subhook Segments (μmol/g fresh wt)*

Uptake after 30 min in 50 mM KCl (pH 6.5) 25 C. Subhook segments: R value significantly lower than D and R/F. Apical hook segments: R value significantly higher than D or R/F.

	Subhook Segments	Apical Hook Segments
D	2.09 (±0.15)	3.90 (±0.27)
R	1.55 (±0.18)	4.41 (±0.18)
R/F	1.97 (±0.17)	4.15 (±0.14)

segments. These changes are in the same direction as Pfr-mediated changes in K^+ uptake in mung bean hypocotyl segments (2). Pfr-mediated changes in Cl^- transport have been reported in *Samanea* pulvini (19). Much evidence exists to suggest that in the face of large negative electropotentials, anion transport in plant cells is an active process (12). Pfr-mediated changes in the uptake of Cl^- may reflect modulations of an active pump, though more knowledge of individual fluxes in this system is needed before definite statements can be made.

Pfr has been shown to inhibit Pi uptake by mung bean subhook segments measured 1 h after the light treatment (21). Figure 1 shows that R inhibits uptake of Pi [^{32}Pi] measured 10 min following the light treatment. The inhibition was enhanced following a 5-min wash in unlabeled solution and was detectable after a 10- and 30-min wash. A wash at 0 C was given to minimize the incorporation of Pi [^{32}Pi] into organic materials during the wash. It is possible that influx of Pi across the plasmalemma is inhibited by R in subhook segments. In apical hook segments (Fig. 2) R enhanced short term Pi [^{32}Pi] uptake. This enhancement increased following a 3-min wash in unlabeled solution and was maintained after a 10- and 60-min wash. These changes possibly reflect modulations of fluxes across the plasmalemma. Whether these changes reflect changes in active or passive uptake at this concentration is not known. More data on influx and efflux at different external Pi would be useful in this context. Uptake of inorganic metabolites can be regulated by the rate of the metabolic processes in which they are involved (17). It is possible that phytochrome-mediated changes in Pi uptake are linked with changes in phosphate metabolism. Rapid Pfr-controlled changes in phosphate metabolism are known to occur in several systems (*e.g.* 7).

Pfr inhibited H^+ extrusion by subhook segments and enhanced extrusion by apical segments in 1 mM KCl (Table II). These changes were generally detectable 1 h following the light treatment. Complete photoreversibility by F was not found in apical segments. Lürssen (11) showed that pea epicotyl segments in 1 mM KCl caused deacidification, especially in solutions of pH lower than 6.0. R caused an increase in deacidification and F following R reversed the effect. In the presence of auxin, acidification occurred and this was inhibited by Pfr. Pike and Richardson (16) reported a small R/F-reversible increase in H^+ extrusion by 1-cm apical oat coleoptile segments in 1 mM KCl. The increase was detected 1 h following the light treatment. The changes in H^+ extrusion reported here are in the same direction as Pfr-mediated changes in K^+ uptake reported previously (2). It is possible that Pfr may control a K^+-H^+ exchange process (6) and it would be of interest to investigate this possibility further. Preliminary experiments using a surface contact method (5) with subhook segments indicate that Pfr does not induce rapid changes in H^+ extrusion. In lower (1 mM) [K^+], Pfr-mediated changes in H^+ extrusion follow a similar time course to changes in K^+ uptake. K^+ uptake/H^+ extrusion, however does not show a 1:1 stoichiometry and only part of K^+ influx can be related to H^+ efflux.

It is clear from these results and those presented previously (3) that phytochrome can control complex changes in short term transport processes. Whether any one of these processes described is directly linked to an initial action or whether they are secondary results is not known.

The differential responses of the two regions of the hypocotyl hook to the same stimulus described here are of great interest. Obviously the state of differentiation of the tissue is important (15). Concentrations of metabolites, enzyme activities, location of phytochrome, composition of membranes, intrinsic transport rates, and other factors may all combine to produce an enhancement or an inhibition of a process responding to the same external stimulus in different cells. The involvement of hormones in these responses

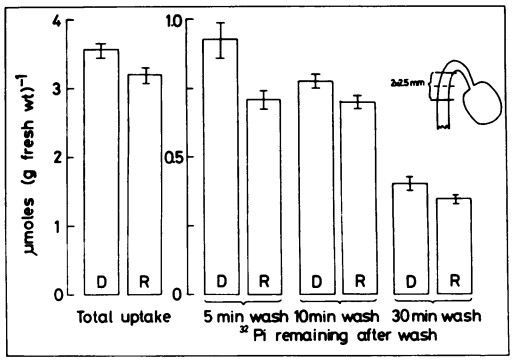

FIG. 1. Effect of R on uptake of Pi [^{32}Pi] by subhook segments in 50 mM K_2HPO_4/KH_2PO_4 buffer (pH 6.5), 25 C, followed by 5-, 10-, and 30-min wash in identical unlabeled solution, 0 C. R values significantly lower than corresponding D.

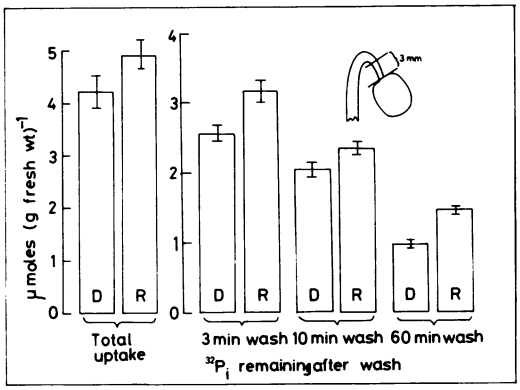

FIG. 2. Effect of R on uptake of Pi [^{32}Pi] by apical hook segments in 50 mM K_2HPO_4/KH_2PO_4 buffer (pH 6.5), 25 C, followed by 3-, 10-, and 60-min wash in identical unlabeled solution. R values significantly higher than corresponding D.

Table II. *H^+ Extrusion by Sub- and Apical Hook Segments in 1 mM KCl (pH 6.5) 25 C (μmol/g fresh wt)*

Extrusion after 1 and 2 h in D or in D following R or R/F. Subhook segments: R values significantly lower than D or R/F. Apical hook segments: R values significantly higher than D but not R/F.

	Subhook Segments			Apical Hook Segments		
	D	R	R/F	D	R	R/F
1 h	1.86 (±0.20)	1.18 (±0.12)	1.50 (±0.13)	1.62 (±0.25)	2.31 (±0.20)	2.12 (±0.14)
2 h	2.53 (±0.21)	1.54 (±0.12)	1.87 (±0.13)	2.38 (±0.14)	2.85 (±0.13)	2.54 (±0.21)

must also be considered. Hormones have been shown to be involved in several photomorphogenic responses (*e.g.* 18, 24) and it is well known that they can influence transport (23). It has been shown (9) that different regions of the bean hypocotyl have different sensitivities to applied hormones.

The changes described in this and preceding papers are in the same direction as the differential responses of growth to Pfr in other systems (10, 15, 22). Such studies have led to the conclusion that Pfr enhances the growth of young cells and inhibits growth of older cells. The possibility arises that the Pfr-mediated changes reported here are consequences of growth changes. This, however, is unlikely as most growth changes in response to R are not detectable within the first 15 min after irradiation. Meijer (14) detected growth inhibition by R only after 30 min. Weintraub and Lawson (25), however, showed R-mediated changes in growth of intact and excised oat coleoptile tissue detectable 1 min following the light treatment. A growth response may also occur before changes in growth are detectable. Bertsch and Hillman (1) demonstrated that sucrose was necessary for a R/F-reversible growth response in isolated pea stem segments. The segments used in this study were preincubated in the absence of sucrose, though sufficient sucrose may be stored in the tissue. The lack of a direct correlation with growth is also implicit from low temperature experiments in which Pfr-mediated change in K^+ uptake persist (3). Furthermore, preliminary experiments measuring growth of subhook segments microscopically have shown no rapid R-mediated changes in extension growth.

Steward and Mott (20) have suggested that ion transport is only one facet of a more general process, *i.e.* the total solute relations of cells. Changes in ion transport can lead to changed turgor relations of the vacuole and activity of water in the cytoplasm. Steward and Mott stress the possible role of K^+ in this context. It is possible that Pfr-mediated changes in ion fluxes bring about changes in the osmotic properties of the cells resulting in changed turgor and growth. This situation may be analogous to the massive Pfr-mediated fluxes of K^+ and Cl^- resulting in changed turgor and leaflet movement in *Samanea* and *Albizzia* (8).

This work demonstrates that modulations of ion transport are early consequences of phytochrome photoconversion. Although these changes precede any detectable growth, only further experimentation will reveal if they have a direct causal relationship leading to changes in growth and development.

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CORRECTIONS

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Pages 206 and 211, titles should be corrected to read: "Ion Fluxes and Phytochrome in Mung Bean Hypocotyl Segments."