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Red Light and Auxin Effects on ^{86}Rubidium Uptake by Oat Coleoptile and Pea Epicotyl Segments[1]

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ABSTRACT

Apical segments of etiolated oat (*Avena sativa* L. cv. Victory) coleoptiles showed enhanced uptake of [$^{86}Rb^+$] when tested 30 minutes after a 5-minute red irradiation. The response was partly reversible by far red light. Uptake was sensitive to carbonyl cyanide *m*-chlorophenyl hydrazone, but not to isotonic mannitol. Indoleacetic acid (10^{-7} molar) caused a very pronounced and rapid stimulation of uptake. Basal coleoptile segments also exhibited a red light-enhanced uptake, but not an effect of red light on changes in the pH of the medium. The [$^{86}Rb^+$] uptake of third internode segments from etiolated peas (*Pisum sativum* L. cv. Alaska) was not affected by either red light or auxin. This tissue also showed no red light effect on acidification of the medium. It is concluded that alteration of [$^{86}Rb^+$] flux is not a general feature of phytochrome action.

The ability of phytochrome to interact with or alter the properties of membranes has been extensively studied (14). Massive fluxes of K^+ and Cl^- between the dorsal and ventral pulvinus motor cells are responsible for leaflet movement in *Albizzia* and *Samanea* (22). Phytochrome-mediated changes in [$^{86}Rb^+$] uptake by mung bean hypocotyl segments were observed within 10 min of irradiation (4). However, Kendrick and Hillman (12) found that R[2] had no effect on the K^+ content of pea epicotyl segments. Light-induced changes in transmembrane ion fluxes are also reflected in measurements of extremely rapid depolarizations of the electrical potential (17).

We have detected a small R-induced acidification of the medium by apical oat coleoptile segments (16). This report describes investigations of the influence of irradiation and of IAA on [$^{86}Rb^+$] uptake by oat coleoptile and pea epicotyl segments, as part of our comparative study of the mechanisms of action of these stimuli. In most tissues (6, 19), but not all (11), [$^{86}Rb^+$] is a suitable label for K^+; since no information is available on the tissues we studied, we refer specifically to "[$^{86}Rb^+$] uptake."

MATERIALS AND METHODS

Etiolated oat seedlings (*Avena sativa* L. cv. Victory) were grown as described earlier (16). Following abrasion of the cuticle with emery powder, apical 1-cm segments (with primary leaf) were cut (16). One set of experiments involved 1-cm segments cut from the base of the coleoptile.

The following general procedure, adapted from the methods of Epstein *et al.* (7), was used for uptake experiments. Some variations are described under "Results." Incubations were conducted in a shaking water bath at 27 ± 1 C. All solutions were aerated. Most experiments involved set A solutions (Table I). The segments (usually 30 per treatment) were enclosed in white nylon mesh "teabags," which were placed in 500 ml of holding solution for 30 min. Then the teabags were washed for two 30-sec periods in 250-ml portions of holding solution and transferred to uptake solution. Each experimental trial involved two treatments with two samples each; 300 ml uptake solution was used for each treatment. Irradiations (16) were usually performed at the beginning of the uptake period. After a variable period in unlabeled uptake solution, the teabags were transferred to [$^{86}Rb^+$]-containing uptake solution (200 ml per treatment, containing about 10^4 cpm/ml) for 15 min. The ^{86}RbCl was obtained from New England Nuclear. Then, all of the teabags were briefly immersed in five successive 250-ml portions of cold desorbing solution and placed in 2.5 liters cold desorbing solution for 30 min. All manipulations to this point were carried out under dim green safelights (16).

The tissue was transferred to scintillation vials, the dry weight determined, and 20 ml of 5 mM 7-amino-1,3-naphthalenedisulfonic acid monosodium salt (Eastman) was added to each vial. Radioactivity was determined by Cerenkov counting (13), using program 1 of a Searle Analytic model 6868 liquid scintillation counter. Efficiency of counting was about 40%. There was little sample to sample variation in quenching, so the results were expressed as cpm/g dry weight tissue, and the values for duplicate samples averaged. There were generally at least three replicates of each experiment.

In experiments with peas (*Pisum sativum* L. cv. Alaska, W. Atlee Burpee), 1-cm third internode sections were cut 3 mm below the apical hook of 7-day-old etiolated seedlings grown in ¼ strength Hoagland solution (1). Sections were either scrubbed with emery powder, cut into 500-μm slices on a microtome, or "peeled" with forceps. Acidification of the medium by pea epicotyl segments and basal oat coleoptile segments was determined as described before (16).

RESULTS

Using a 30-min period in unlabeled uptake solution (with irradiation at the beginning), followed by transfer to labeled uptake solution for the standard 15-min period, we observed a considerable stimulation of uptake by R (Table II). The data are expressed on a per cent basis in each pair of samples. FR gave some stimulation of uptake compared to dark (Table II), but the effect was less than half that of R. Also, there was only a partial reversal of the red effect by FR (Table II). No stimulation of uptake by R could be detected when the irradiation was given at the beginning of the 15-min period in labeled uptake solution (data not shown). When experiments were conducted in either solution set A or B, using a 1-hr period in unlabeled uptake solution, there was also stimulation of uptake by R (data not shown).

In experiments with CCCP and mannitol, all samples received

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[2] Abbreviations: R: red light; FR: far red light; CCCP: carbonyl cyanide *m*-chlorophenyl hydrazone.

Table I. Solutions for [$^{86}Rb^+$] Uptake Experiments

	Set A	Set B
HOLDING		
	1 mM MES-TRIS 0.5 mM $CaCl_2$ pH 6.5	0.5 mM $CaCl_2$ pH 5.9
UPTAKE		
	1 mM MES-TRIS 0.5 mM $CaCl_2$ 1 mM KCl pH 6.5	0.5 mM $CaCl_2$ 0.2 mM RbCl pH 5.9
DESORBING		
	10 mM KCl	10 mM KCl

Table II. [$^{86}Rb^+$] Uptake by Etiolated Oat Coleoptile Segments Following Irradiation

The procedures for measuring ion uptake are described in the text. Except where noted, a 30 min period in unlabelled uptake solution preceded the 15 min period in label. In each experiment the uptake (cpm/gm dry weight) of the treatment to the right of the slash was set at 100%. The uptake of the treatment to the left of the slash is expressed as a %. Data are expressed as mean ± SE.

Treatment	[$^{86}Rb^+$] Uptake %
5 min Red/Dark	134 ± 5
5 min Red/Dark (2 hr in unlabelled uptake solution)	96 ± 2
5 min Red/5 min Red + 5 min Far Red	108 ± 2
5 min Far Red/Dark	115 ± 2
5 min Red + 3 x 10^{-6}M CCCP/5 min Red	6 ± 1
5 min Red + 0.45M mannitol/5 min Red	97 ± 5

R; the test substances were added to one portion of the uptake solutions (both unlabeled and labeled). CCCP, at 3×10^{-6} M, reduced uptake by 94% compared to the control level (Table II). Mannitol, at 0.45 M, had essentially no effect.

As shown in Table III, 10^{-7} M IAA (present in both unlabeled and labeled uptake solution) induced a doubling of [$^{86}Rb^+$] uptake compared to the control, when there was a 30-min period in unlabeled uptake solution. When the period in unlabeled uptake solution was eliminated, and IAA and [$^{86}Rb^+$] were given together for 15 min, there was still a substantial promotion of uptake by the hormone. The latent period of the IAA effect is less than 15 min. When IAA and R were given together at the beginning of the 30-min period in unlabeled uptake solution, the uptake was not significantly different from the IAA-only treatment (Table III).

Substantial amounts of uptake by pea epicotyl segments were obtained only when the holding period was lengthened to 20 hr (12, 19) and tissue slices were prepared in order to increase the surface area (19). We tested unlabeled uptake periods ranging from 0 to 2 hr and tested both sets of solutions. Uptake was always severalfold greater when set B was used. Because the R inhibition of pea stem segment growth *in vitro* is dependent on the presence of sucrose and cobalt in the incubation medium (2), experiments were also carried out using solution set B with 1.5% sucrose and 2×10^{-5} M $Co(NO_3)_2$ in the holding and uptake solutions. In no case was the mean uptake value for the R-treated tissue more than a few per cent different from the dark control value (data not shown), so we conclude that R had no effect on [$^{86}Rb^+$] uptake in pea epicotyl tissue. We also found no effect of 10^{-7} M IAA on uptake by this tissue (data not shown).

We next investigated the effect of irradiation on acidification of the medium by abraded pea epicotyl segments. Three hr after irradiation, in a typical experiment the change in pH of the dark control samples was −0.36 and of the R-treated sample, −0.37. No differences were detected at shorter intervals after irradiation or using peeled segments in several different media.

Basal oat coleoptile segments (scrubbed with emery powder) caused an increase in pH of the medium. There was no indication

Table III. [$^{86}Rb^+$] Uptake by Etiolated Oat Coleoptile Segments Treated with IAA

The procedures for measuring ion uptake are described in the text. Except where noted, a 30 min period in unlabelled uptake solution with IAA preceded the 15 min period in label. In each experiment the uptake (cpm/gm dry weight) of the treatment to the right of the slash was set at 100%. The uptake of the treatment to the left of the slash is expressed as a %. Data are expressed as mean ± SE.

Treatment	[$^{86}Rb^+$] Uptake %
10^{-7}M IAA/Buffer	203 ± 1
10^{-7}M IAA + 5 min Red/10^{-7}M IAA	96 ± 1
10^{-7}M IAA + 5 min Red/5 min Red	164 ± 3
10^{-7}M IAA/Buffer (no unlabelled uptake period)	122 ± 4

of a significant light effect; in a typical experiment, the pH changes measured 2 hr after irradiation were: dark, +0.29; 5 min R, +0.25; 5 min R + 5 min FR, +0.31; 5 min FR, +0.29.

Ion uptake by basal coleoptile segments was studied using solution set A and a 30-min period in unlabeled uptake solution. The [$^{86}Rb^+$] uptake by basal dark control segments was far less than that of apical segments (for example, 15,900 and 93,700 cpm/gm tissue, respectively). There was a stimulation of uptake by 5-min R (120% of the dark control value). The stimulation was about half reversed by 5-min FR (R-treated compared to R + FR, 112%), and 5 min FR alone had a small effect (107% of the dark control value).

DISCUSSION

R stimulated [$^{86}Rb^+$] uptake by apical oat coleoptile segments, in experiments with a pulse of label following an unlabeled uptake period. No promotion was seen if the R was given at the beginning of the 15-min labeled uptake period. The lag period is no more than 30 min (Table II), although we did not explore this point further. FR alone yielded some stimulation of uptake compared to the dark control, but the effect was less than half that of R (Table II). It is possible that the small per cent Pfr established in FR is sufficient to trigger some enhancement of [$^{86}Rb^+$] uptake. The incomplete reversal of the R effect by FR could also be explained in this way. Also, the response might have a very rapid escape from photoreversibility. A pigment other than phytochrome might be involved, as suggested for similar FR responses in growth studies of intact oat coleoptiles (3).

The almost complete elimination of ion uptake by CCCP suggests that the uptake is energy-dependent. Incubation in a plasmolyzing concentration of mannitol did not interfere with the uptake mechanism. The [$^{86}Rb^+$] uptake induced by fusicoccin in subapical coleoptile segments was also strongly inhibited by CCCP, but, in contrast, was 30% inhibited by mannitol (5).

Several comparisons can be made between these results and our findings on acidification of the medium (16). The R stimulation of [$^{86}Rb^+$] uptake was seen much more rapidly than the stimulation of acidification (which was measured 2 hr after irradiation). When a 2-hr period in unlabeled uptake solution was used, the R-irradiated samples showed 96 ± 2% of the uptake of the dark control (Table II). There is no effect of R on uptake at a time when the R effect on acidification is quite evident. Of course, the media used to study ion uptake and acidification are different, so we performed [$^{86}Rb^+$] uptake experiments using the 1 mM K-phosphate buffer (pH 6.2), of our acidification work as the holding and uptake solution. Again, R stimulated [$^{86}Rb^+$] uptake using a 30-min period, but not a 2-hr period, in unlabeled uptake solution. Therefore it is unlikely that R is promoting an electroneutral $K^+ - H^+$ antiport (9). Mechanisms such as stimulation of electrogenic pumps should be considered (5). The R effect on acidification is more fully reversible by FR. Both processes are strongly inhibited

by CCCP, but the pH effect is promoted by mannitol, which has no effect on [$^{86}Rb^+$] uptake.

We found a rapid effect of 10^{-7} M IAA on [$^{86}Rb^+$] uptake. The stimulation was detectable within 15 min, and, when a 30-min unlabeled uptake period was used, IAA induced a doubling of uptake. Cleland (5) reported a longer lag and a less pronounced effect; there are, however, several differences in the methods of growing plants and preparing segments (16). Rubinstein and Light (21) showed that IAA enhanced [$^{36}Cl^-$] uptake by subapical oat coleoptile segments (epidermis intact) within 15 min, although the peak promotion was much less than that seen in the present work.

Our results indicate that IAA acts much more rapidly and strongly than R on [$^{86}Rb^+$] uptake by apical oat coleoptile segments; the same pattern applies to the stimulation of acidification (16, 20). When R and IAA were applied together, the promotion of [$^{86}Rb^+$] uptake was not different from that caused by IAA alone. Perhaps the large activation of transport by the hormone has precluded any further activation by R; complete dose response curves would be needed to test this hypothesis.

The R effect reported occurs with a much longer lag than the change in potential (17), so the potential change is not a side effect (14) of the change in ion flux. Similarly, in the *Samanea* pulvinus the phytochrome-mediated depolarization of the transmembrane potential occurs considerably before the massive K^+ flux, and other membrane processes may be responsible for the potential change (18). Measurements of uptake from the external solution would not detect movement of ions within a tissue, but microelectrode measurements can indicate changes in potential resulting from such fluxes.

Pea epicotyl tissue was studied because its growth response to R is opposite that of apical oat coleoptile segments (10). No R effects on [$^{86}Rb^+$] uptake were detected. Using flame photometry, Kendrick and Hillman (12) could not detect any change in the K^+ content of pea epicotyls, measured 3 hr after irradiation. In contrast, R had a very rapid effect on [$^{86}Rb^+$] uptake by mung bean hypocotyl segments (4).

We found no effect of 10^{-7} M IAA on [$^{86}Rb^+$] uptake by pea epicotyl tissue. IAA induces a hyperpolarization of the membrane potential within 1 hr (15) and proton efflux within 20 min (20), but neither of these processes seems to depend on an IAA-induced change in [$^{86}Rb^+$] flux.

Basal oat coleoptile segments were tested as a system which does not exhibit growth promotion by R. In a 24-hr growth test, R-treated segments (1 cm) grew slightly (but not significantly, by the t test at $P = 0.05$) less than dark control segments. The ion uptake was appreciably less than in apical segments, and there was a smaller R stimulation of uptake. Thus, there is evidence for phytochrome influence on [$^{86}Rb^+$] uptake in both regions of the coleoptile, although their growth responses are different. It is unlikely that the uptake of [$^{86}Rb^+$] is a direct part of the chain of communication between phytochrome and the growth-controlling system. Alternatively, effects on uptake and on growth may represent distinct aspects of phytochrome action. In contrast to the R promotion of acidification in apical segments (16), basal segments did not show an effect of R on the pH of the medium.

The alteration of [$^{86}Rb^+$] uptake is not a standard feature of phytochrome action, nor does R always influence the ability of tissue segments to alter the medium pH. Likewise, not all tissues may exhibit phytochrome-mediated changes in electrical potential (8).

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