Short Communication

Phytochrome Modifies Blue-light-induced Electrical Changes in Corn Coleoptiles

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ABSTRACT

Unilateral blue light administered to corn coleoptile segments produces no alteration of transmembrane potential on the light side, and only a small and slow hyperpolarization on the dark side. Red light causes a 5-15 millivolt depolarization in cells on the light side causes and somewhat smaller effects on the dark side. Blue given after red causes a rapid hyperpolarization on both sides of the coleoptile. The effect of the potentiating red preradiation is probably due to phytochrome, being largely abolished by far-red given after red, but before the blue light. The effect of prior red irradiation decays in the dark, showing a half-time of about 45 minutes at room temperature. This rapid cooperativity between phytochrome and the phototropic pigment may indicate a common locale, possibly in a membrane.

The phototropic responses of coleoptiles to blue light are affected by pretreatments with other wavelengths of light (1). In corn coleoptiles, prior red radiation reduces the sensitivity to blue light, but increases the extent of curvature (2, 4). Far-red light given immediately after red reverses these effects, suggesting that phytochrome is involved, and capable of interacting in some way with the receptor for phototropism. The red light-induced reduction in phototropic sensitivity lasts for about 1 h in the dark, at which time the normal sensitivity to blue light is restored. Similarly, preradiation of etiolated pea seedlings with red light promotes the subsequent bending response of the epicotyl to blue light (9). Blue light also induces a photobleaching of presumed flavin-containing pigments in membrane segments derived from pea epicotyl extracts and red light may influence the nature of these spectral changes as well (5).

In this paper, we report on electrical responses of coleoptile cells to treatments with blue light, with and without prior red or far-red irradiation. Inasmuch as electrical measurements can provide an instantaneous and continuous record of membrane-based events in living cells, observation of such potentials under different light conditions may reveal events close to the primary site of light action.

MATERIALS AND METHODS

Corn seedlings (Zea mays, var. Golden Bantam) were grown for 5 days in the dark at 25 C and about 80% RH. Coleoptiles were excised by gently withdrawing the apical 5 cm from the inner true leaf. Coleoptile tissue, mounted in a Plexiglass holder fixed in a chamber adapted for continuous flow of solutions (10), was incubated in darkness and perfused for 6 h with aerated 1X solution (8) before use in the experiments. After this, the entire chamber was mounted on the micromanipulator stage of the microscope and viewed at 250X with a morphogenetically inactive green safelight (6). Unilateral light was administered to the coleoptile by a 2-mm diameter fiber optic passing through the end of the chamber and positioned within 500 mu of the coleoptile tissue. Blue light with a peak of 454 nm and a band width of 25 nm was obtained by filtering tungsten filament microscope lamp with an interference filter (Oriel Corp., Stamford, CT, No. G-572-4600) and a long wavelength absorption filter (Oriel Corp., No. G-774-4050). The intensity of blue light emerging from the filter pack was about 300 mu/cm²; the intensity after passing through 30 cm of the fiber optic tube could not be accurately determined. Red and far-red wavelengths of 650 nm and 730 nm, respectively, were obtained with filter combinations that have previously been described (11).

Simultaneous, electrical measurements from individual cells on opposite sides of the coleoptile were made by advancing salt-filled glass microelectrodes through the epidermal cell layer into the underlying cortical cells. Visibility of the electrode tip during this procedure was sufficient to tell which cell was being impaled although it was impossible to ascertain whether the electrode was in the cytoplasm or vacuole. As is becoming customary for electrical measurements in small cells of higher plants, we considered the electrode to be in the large, central vacuole when measurements were being made. The protocol for amplification and recording of signals is fundamentally unchanged from that which we have reported elsewhere (10).

RESULTS AND DISCUSSION

Both unilateral red and blue light produced rapid changes in the membrane potential of corn coleoptile cells. In tissue exposed to a 30 s unilateral blue light without a red light pretreatment, the membrane potential changed little in the cells on the light side, but slowly hyperpolarized on the darkened side (Fig. 1a). When a 5-min red light pretreatment was given, the voltage responses in cells on both sides of the coleoptile were very different. Red light alone caused a 5-15 mv depolarization in cells on the light side and a somewhat smaller depolarization (2-10 mv) on the darkened side (Fig. 1b), while subsequent irradiation with 30 s of blue light caused a rapid increase in membrane potential in cells on both sides of the coleoptile (Fig. 1c). This response to light treatments had a more complex waveform than the slow response but the maximum hyperpolarization in both non-pretreated cells and red light pretreated cells was nearly the same after about 20 min. Two min after red light, given after red light abolished the rapid blue-mediated response, revealing again the slow increase in potential in cells of the darkened side of the coleoptile (Fig. 1d). The effect of a 5-min pretreatment of red light on the blue light voltage

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responses was diminished if a dark period of 30–45 min intervened between red and blue light (Fig. 2). This compares favorably with the delay time for red light enhancement of the phototropic response (2).

Although it is hazardous to propose a causal relationship between these light induced voltage changes and the complex processes of lateral auxin transport and coleoptile bending, we are certain that the blue light irradiations which produce electrical changes also induce bending of the coleoptile. Coleoptiles which were left in the perfusion chamber following impalement with microelectrodes exhibited positive phototropic bending of at least 10°, within 90 min of irradiation with unilateral blue light. Because we were unable to measure the intensity of light at the coleoptile, it is not known if the bending was first or second positive phototropism.

There have been two other reports that are related to this effort to correlate electrical measurements and auxin responses. Graham (7) measured external voltages on coleoptiles using surface electrodes and found that voltage changes following unilateral irradiation occurred after the arrival of auxin at the measured sites. More recently, Cleland et al. (3) showed that exogenously applied auxin hyperpolarized oat coleoptile cells.

The measurements reported here show that cellular voltage responses are asymmetrical in coleoptiles not pretreated with red light, and become more rapid and symmetrical in the red-light pretreated tissues. The greater rapidity of the response to blue light following the red light pretreatment may be correlated with the enhancement of final coleoptile curvature that one sees under these light conditions. The equivalent voltage response of both light and dark side cells in the red-pretreated tissue may relate to the observed loss in blue light sensitivity.

Since the voltage responses occur within seconds to minutes after irradiation, it is likely that an early consequence of the absorption of phototropically active light is to alter the properties of cell membranes. Since the primary site of auxin activity is also thought to be the cell membrane (3), it is perhaps logical that the interacting processes of photoreception and hormone-induced elongation are coordinated at the membrane level.

LITERATURE CITED

2. BRIGGS, WR, HP CHON 1966 The physiological versus the spectrophotometric status of phytochrome in corn coleoptiles. Plant Physiol 41: 1159–1166

FIG. 1. Voltage recordings from corn coleoptile cells in different light regimes. Recordings on the left are from cells proximal to the fiberoptic-light source; those on the right are from cells distal to the light. All recordings are redrawn, with compressed voltage and time scales, from representative measurements of coleoptile cells. Gaps in the recordings are 3-min periods in which the potential did not change by more than 1 mV.

The results are from experiments that were repeated at least 6 times with similar results. Absolute membrane potentials in these experiments ranged from -115 to -160 mV. Three control experiments that were done are not included in these records: The red light treatment caused a 5–10 mV depolarization which did not repolarize after 20 min. Far red light treatments or darkness produced no changes in voltage in cells on either side of the coleoptile after 20 min.

FIG. 2. Changes in the kinetics of blue-light induced voltage responses in "dark side" coleoptile cells following a 5 min red light pretreatment and a dark period of increasing duration. Cells showing either a "slow" (greater than 5 min) hyperpolarization of the membrane in blue light (Fig. 1a) or a "fast" (2 min or less) hyperpolarization (Fig. 1c) are represented as a percentage of the total number of cells measured. In nearly all cases the cells responded with either "fast" or "slow" kinetics; thus the percentage of fast responses added to the percentage of slow responses equalled 100%. However, with a 45 min dark period between red and blue light, about 27% of the cells showed either a transient (less than 30 s) change or no change in potential. Data are presented as avg. ± 1 SD of at least 10 determinations.

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