Light-induced, Dark-reversible Absorbance Changes in Roots, Other Organs, and Cell-free Preparations

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ABSTRACT

Irradiation of maize (Zea mays) roots and coleoptiles with visible light causes dark-reversible absorbance changes in these organs. There is an increase in absorbance near 440 nanometers and smaller increases below 410 nanometers and about 595 nanometers. Decreases in absorbance are observed at about 420 nanometers and minor ones at 537 and 575 nanometers. These responses are also observed in cell-free preparations from roots and coleoptiles if dithionite, NADPH, or NADH is added prior to illumination. The dose curve for these effects has a distinct maximum at 420 nanometers and a minor one at 575 nanometers. Difference spectra and dose response curves indicate that these compounds such as cytochromes or, more probably, peroxidase complexes are the photoreceptive and chemical reacting molecules. Siroheme-containing proteins may also be taken into consideration.

The light-induced absorbance changes have half-lives of more than 200 seconds and 100 seconds in roots of maize and soybean, respectively. Two reactions, each with first order kinetics, appear to be superimposed. The respective rate constants for maize roots are about 0.004 and 0.04 seconds⁻¹. The generation of the effect has a much shorter half-life dependent on light intensity and wavelength. Little deviations from first order kinetics were detected. Rate constants for corn roots range between 0.05 and 0.01 seconds⁻¹.

Apart from the problem in which hemoproteins are involved, there is the problem of correlating the reaction of the photoreactive and chemically reacting molecules to macroscopic responses such as phototropism.

Some of the most remarkable biological phenomena, other than photosynthesis, are developmental mechanisms regulated by light. Biosynthetic pathways, morphogenesis, and movements of plants are influenced by visible radiation.

Phytochrome is the best known photomorphogenetic pigment. The links between absorption by this pigment and morphogenetic changes have not been identified. There are a number of other photobiological phenomena not involving phytochrome for which the photoreceptor(s) have not been identified clearly, e.g. phototropism, phototaxis, carotenogenesis, O₂ uptake. Because the spectral response curves of all of these responses have a maximum at about 460 nm (6), carotenoids or flavins have long been considered the photoreceptors in these cases. Recent results obtained with Dictyostelium discoideum (8) and Neurospora crassa (6) favor flavins rather than carotenoids. Spectral A changes caused by photoreduction as well as photooxidation of Cy are reported to be triggered by flavins (9, 10).

Little is known about similar responses in higher plants although phototropism has been studied extensively for almost a century. During our studies of light-induced A changes in higher plants we found only two reports on this subject: one comparing a particulate fraction from corn with a similar one from fungi (3), the other dealing with coleoptiles of wheat (13).

Roots of corn which show negative and positive phototropism as well as light-induced geotropism (11) were chosen for our first experiments. We describe here reversible absorbance changes in intact roots and coleoptiles of maize as well as in cell-free preparations from these tissues.

MATERIALS AND METHODS

Seedlings of Zea mays (WFG TMS × BS7 or FR_{50B} × FR_{37}, Illinois Foundation Seed Co.) were grown in light or darkness in Vermiculite, in plastic dishpans. Roots were harvested after 5 to 7 days of growth at 25 C. Three-cm tip sections excised from roots grown in the space between the Vermiculite and the walls of the dishpan were used for the experiments. Only sturdy, bright white roots were taken. Roots of other species as well as coleoptiles of dark-grown maize were also studied. Coleoptiles were excised when they were 4 to 5 cm long; the primary leaves were removed before the 2-cm tip section was used in the experiments.

Roots and coleoptiles were cut into 1- to 2-mm sections under nutrient solution. This solution contained 10⁻³ M NH₄Cl, 10⁻³ M MgSO₄, 5 x 10⁻⁴ M CaCl₂, and 2 x 10⁻³ M K₂HPO₄. Thereafter, the chopped material was transferred to cuvettes (1-cm light path) also filled with nutrient solution. Experiments were also performed with tissues submerged in H₂O. The A of a sample was about 2.0 to 2.5 at 720 nm.

Light-dark difference spectra and the kinetics of light-mediated A changes were measured with a split beam, double monochromator Aminco DW 2 spectrophotometer. The cuvette compartment of the spectrophotometer had covered openings through which a beam of actinic light could be applied perpendicular to the photometer beams via light pipes. It also contained a device to insert filters into the actinic beam. In kinetic measurements using yellow actinic light, stray light was prevented from entering the phototube by a combination of filters (two Balzers DT Blau, one Perkin-Elmer Short wave pass No. 904). The phototube was shut down during actinic illumination with other wavelengths.

Samples were irradiated with monochromatic light generated by a Bausch & Lomb monochromator (1350 grooves/mm) and a mercury light source SP 200 in combination with interference filters, or a high intensity illuminator model 170-D (Dolan Jenner Corp.) and interference filters (half-bandwidth 15 nm).

An ISCO model SR spectroradiometer calibrated by means of an ISCO model SRC and a Kipp & Zonen, Holland thermopile (for wavelengths below 420 nm) in combination with a galvanometer were used for measuring light intensities. The source intensi-

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ties were adjusted to the same quantum flux by changing the distance between the mercury light source and the entrance to the light pipe or by changing the voltage of the high intensity illuminator. Light intensities are those at the outer surface of the cuvette. The measuring beams were too weak to influence the sample. All experiments were performed at room temperature.

Cell-free extracts of root and coleoptiles were prepared by grinding tissues with glass beads in a precooled mortar provided with grinding medium (tissue/medium = 1/6). The grinding medium was composed of 15 g of sucrose in 100 ml of nutrient solution which was also 10^{-4} \text{M Tris-HCl (pH 7.5)} and 5 \times 10^{-3} \text{M mercaptoethanol}.

The slurry was filtered through one layer of cloth and centrifuged for 5 min at 500g. The supernatant fluid was used in the experiments. Particle-free supernatants were prepared by additional centrifugation in a Spinco centrifuge at 5 C at 80,000g for 2 hr.

**RESULTS**

**Effect with Intact Root Tips.** Two samples of almost equal A were prepared by slightly compressing or releasing the root cylinders in the cuvettes. Small remaining differences were eliminated by means of the spectrophotometer's trim board. A changes could be detected after the sample was illuminated for a few sec. There was an increase in A at 439 nm and a very small one at about 595 nm; decreases occurred at 419, 537, and 575 nm. Figure 1 shows typical light-dark difference spectra in the blue and the green-yellow regions. The ratio of deviations from the dark-light line at 419 and 439 nm was about 100:58. The deviations in the green-yellow regions were much smaller. The respective ratios comparing the changes at 537 and 575 nm with those at 419 nm were 10:14:100. All changes caused by light were reversed subsequently in darkness.

The magnitude of the response increased within an hr after harvest and remained more or less constant during the following hr of experimentation. Our experiments were performed during the constant phase.

Root sections far from the tip (>5 cm) rarely showed any effect.

**Kinetics.** Half of the A change, measured as the absolute difference between the A at 419 and 439 nm, decayed in about 100 (103 ± 8) sec. Subsequent periods during which half of the respective remaining A changes disappeared lasted an average of about 165 sec. The average values of 17 experiments were 153 ± 11, 164 ± 10, and 173 ± 13 sec for the second and the following periods, respectively. The biphasic nature of the decay is seen clearly in the semilogarithmic plot in Figure 2b of the spectrophotometric data of Figure 2a. A fast and a slow reaction, each with first order kinetics, are superimposed. The rate constants are about 0.04 sec^{-1} and 0.004 sec^{-1}, respectively.

The A changes increased more rapidly in the light than they decreased in darkness. Half-lives depended on light intensity and wavelengths. A typical time course for development and disappearance of the A changes is given in Figure 2a. In yellow light (575 nm) a half-life of about 65 sec was measured at an intensity of 4 \mu W/cm^2-nm and about 15 sec at about a 20-fold higher intensity. The relation of half-life to intensity of illumination is given in Figure 3a. In contrast to the situation found when measuring the reversal of the effects, only minor deviations from first order kinetics were detected. Rate constants are of the order of 0.05 to 0.01 sec^{-1}. In the experiment shown (Fig. 2a) the rate constant is about 0.02 sec^{-1}.

The magnitude of the effect increased with all intensities tested although it appeared to level off at about 70 \mu W/cm^2-nm (Fig. 3b). Exposure to lower intensities resulted in steady-state conditions below the maximum. Exponential curves similar to those given in Figure 3b were obtained when the magnitude of the response was plotted versus the time of illumination (Fig. 3c).

**Equal Dose Action Spectrum.** Yellow light was not the most effective in evoking A changes but was chosen for these experiments because it does not seem to affect other known light reactions in plants. A dose response curve for the A changes measured as the A difference between 419 and 439 nm shows that there is a high and sharp maximum at 420 nm and a smaller peak with a long slope toward shorter wavelengths at 575 nm. Figure 4 shows an action spectrum obtained with a quantum flux of 19.2 \text{pE/cm}^2-\text{sec} \cdot \text{nm} and 15 sec of illumination. About 70% of the reaction at 420 nm was achieved by increasing light intensity 5-fold in the green-yellow region. Red light, even at much higher intensities, had no effect.

**Cell-free Preparations.** Responses comparable to those already described were observed when cell-free homogenates were prepared from maize roots. Prior to an assay, however, a reductant, such as a few mg of dithionite or NAAD or NADPH to a concentration of 4 \times 10^{-3} \text{M had to be added. Best results were obtained about 30 min after addition of the reductant. Ascorbate and K_2[Fe(CN)]_6} had no promotive effects. The addition of riboflavin phosphate or flavin mononucleotide clouded the light-mediated A changes so that results could not be analyzed. Figure 5 shows the response and its decay in a typical experiment performed with a cell-free homogenate. After obtaining the dark-dark baseline, the sample cuvette was illuminated for 5 sec. The subsequent A changes were scanned at different time intervals.

Generally speaking, the difference spectrum is quite similar to that obtained with root sections; the same is true for the spectral dose response curve (not shown). The peaks of the difference spectrum, however, are shifted to longer wavelengths, from 419 to 422 and from 439 to 443 nm. In addition, a minor broad increase in absorption was observed below 410 nm. Although the A changes were generally reversible in many experiments, there was a small residual negative peak, slowly drifting to higher wavelengths (Fig. 5). Repeated illumination resulted in responses of similar magnitude. (In some experiments the development of new peaks was observed following this drift, negative ones at 428 and 558 nm [minor] and broad positive ones at 468 and 408 nm [minor]. They mostly disappeared during the following hr.)

After the particles in the homogenate were removed by centrifugation at 80,000g for 2 hr at 5 C, an A change could still be
FIG. 2. a: Kinetics of generation and disappearance of light-mediated \( A \) changes in corn roots. Yellow light (20 \( \mu \text{W/cm}^2\) nm, 575 nm) was turned on at upward arrows (L) and off at downward arrow (D). b: Kinetics of decay (\( A', A'' \)) and generation (B) of the 419/439 nm \( A \) change. Values from Figure 2 are replotted on a logarithmic \( A \) scale. Values of \( A' \) indicate differences between measured \( A \) values and those corresponding to solid straight line \( A \). Rate constants of this experiment are about 0.003 sec\(^{-1} \) (A), 0.03 sec\(^{-1} \) (\( A' \)), and 0.02 sec\(^{-1} \) (B). \( \Delta \): \( A \) change.

FIG. 3. Half-life and magnitude of \( A \) changes in corn roots with respect to light quantity. Magnitude was measured as difference between \( A \) at 419 and 439 nm. a: Dependence of half-life on intensity of yellow light at 575 nm. b: Dependence of magnitude of \( A \) changes on intensity of 575 nm light. Values represent magnitude of response after steady-state level was attained. c: Dependence of magnitude of \( A \) changes on illumination time, shown for blue (430 nm, 5 \( \mu \text{W/cm}^2\) nm = 18.0 pE/cm\(^2\) sec nm) (\( \Delta \)) and yellow (575 nm, 20 \( \mu \text{W/cm}^2\) nm = 96.0 pE/cm\(^2\) sec nm) (C) light.

FIG. 4. Equal dose action spectrum for light-mediated \( A \) changes in corn roots. Equal numbers of quanta were used, corresponding to 4 \( \mu \text{W/cm}^2\) nm (—) and 20 \( \mu \text{W/cm}^2\) nm (—) at 575 nm (19.2 and 96.0 pE/cm\(^2\) sec nm, respectively) and an illumination time of 15 sec.

in particulate homogenates (\( A \) at 720 nm about 1.8). It is doubtful whether the peak at 443 is present; the trough may be found shifted to 412 nm. Some slight activity could be detected in the resuspended pellet.

Coleoptiles, Epicotyls and Roots of Other Species. \( A \) changes similar to those found in roots also occurred in coleoptiles of maize (Fig. 6). \( A \) change maxima, was found at 424 nm (decreases) and 447 nm (increases).

Besides these responses, there was an increase in \( A \) below 410 nm similar to that described in root homogenates. Homogenates of coleoptiles showed \( A \) changes at about the same positions as coleoptile section.

The half-life of the dark absorption change following illumination was more than 200 sec. Minor deviations from first order kinetics were observed. The dose response curve was very similar to that given in Figure 4. In epicotyls of maize (\( A \) at 720 nm about 3.0) only very small effects could be found. The response scarcely attained 1/3 of the response in coleoptiles or roots. In addition, side effects caused by the necessary high doses of blue light obscured the results.

In amaranth coleoptile roots of soybeans, all of the responses...
in cell-free preparations can only be produced if reductants such as dithionite, NADPH, or NADH are added prior to illumination. The decreases in $A$ at two positions in the green-yellow region raise the possibility that Cyt $b$ or $c$ might be oxidized (13). However, the positions of these changes at 537 and 575 nm are quite unusual. Known Cyt $b$ or $c$ have peak positions at wavelengths 10 to 15 nm shorter (2). A heme compound found by Lundegårdh (5) in roots of wheat seems to be a much better candidate. This compound had maxima at 424, 540, and 571 nm and was detectable in roots held anaerobic. It was characterized as a reduced peroxidase complex. The root sections in cuvettes are at least slightly anaerobic, thus the disappearance of the peaks at 537 and 575 nm during illumination, i.e. the appearance of troughs, could indicate a change in such a compound. Corresponding changes should occur in the region of the Soret band. If the $A$ change were solely the consequence of the oxidation of a peroxidase, a trough at about 424 nm and a new peak at about 404 nm should be observable.

The difference spectra, however, do not show such simple relations. The formation of a trough at about 420 nm is always accompanied by a dramatic increase of $A$ at about 440 nm besides smaller ones below 410 nm and eventually about 595 nm. This variety of peaks and troughs suggests that changes in other hemoproteins, e.g. Cyt A (Cyt $a$+ $a_3$) may also be involved. Reduced Cyt A has a maximum in this region and oxidation of the reduced peroxidase complex could cause reduction of Cyt A. Accordingly, the trough at 420 could be ascribed to the combined effects of disappearing reduced peroxidase and disappearing oxidized Cyt A.

However, this interpretation creates a problem. Why would Cyt A remain not fully reduced by NADH, NADPH, or high amounts of dithionite? Could there be a light-mediated shift of electrons within a complex of Cyt A?

Siroheme proteins may also be considered. In the green-yellow region the spectra of reduced and oxidized spinach nitrite reductase (12) show peak positions similar to those observed in the present investigation. However, the correspondence is poor in the Soret region.

An observation of Yokota and Yamazaki (15) may be pertinent. These authors have described the formation of a horseradish peroxidase compound in the presence of NADH or NADPH and O$_2$. This compound has a spectrum similar to that found by Lundegårdh (5). NADH (NADPH) is oxidized and as soon as O$_2$ is consumed the peroxidase compound disappears and a mixture of ferric and ferrous forms of the enzyme is formed. If we assume the formation of a similar compound in corn roots, changes in $A$ caused by light could point toward light-mediated dissociation or destruction of this compound. The ferrous form would be responsible for the increase in $A$ at about 440 nm, the ferric form for the increase below 410 nm. Recombination could take place in the dark. According to our kinetic data this process should be biphasic.

CO hemoproteins have long been known to be light-sensitive (2). Yamazaki et al. (14) reported a red light-sensitive peroxidase compound formed in the presence of dithionite and CO. Although we do not know exactly what kind of complex is present in roots and coleoptiles, we may assume that a similar mechanism is responsible for the spectrometrically detectable changes in these organs.

Apart from the problem of which hemoproteins are involved and where they are located in the cell is the question of whether the $A$ changes in phototropic organs have anything to do with phototropism. Light-mediated changes in the redox state of hemoproteins could influence cell respiration, metabolic pathways, or membrane permeability. Differences in hormone synthesis or growth by cells in a light gradient across an organ can be imagined easily. However, the only data for comparisons are spectral response curves for phototropism and the light-induced absorption changes.

The hemoprotein spectral changes show distinct maxima at 420...
nm and a minor peak at 575 nm. In contrast, light at about 460 nm is most effective in the majority of phototropic responses. One exception is the phototropic base reaction of the coleoptiles. The magnitude of the response increases with decreasing wavelengths from about 500 nm but no distinct maximum has been found (4). More than one photoacceptor could be involved. Phototaxis of Dictyostelium discoideum (6) is reported to be elicited maximally at about 430 and 565 nm, although responses are also obtained at 460 nm (7). Porphyrin-like action spectra are also reported for carotenogenesis in bacteria (1). The 460 nm maximum in phototropism and phototaxis has been ascribed to absorption by flavins. Changes in their redox state during illumination have been linked to reductions or oxidations of Cyt which are hypothesized to affect cell metabolism somehow (9, 10). Our experiments show that light absorbed by the hemoprotein(s) in vivo or in cell-free preparations results in its (or their) oxidation; but in none of these cases are the A changes and oxido-reductions firmly connected causally to phototropism or phototaxis. It will be necessary to compare the responses in respect to intracellular location, energetics, and ubiquity to see whether the different effects are cooperative, antagonistic, or wholly independent.

LITERATURE CITED