Plant Physiol. (1977) 60, 736-738

Specific Inhibition of Phototropism in Corn Seedlings[1]

Received for publication February 14, 1977 and in revised form August 10, 1977

WERNER SCHMIDT,[2] JAMES HART,[3] PHILIP FILNER, AND KENNETH L. POFF[4]
Michigan State University-Energy Research and Development Administration, Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48824

ABSTRACT

Geotropism was used as a control for the specificity of potential inhibitors of phototropism by the coleoptiles of corn (*Zea mays*) seedlings. The compounds tested fall into three categories showing: (*a*) no inhibition of either phototropism or geotropism (KCl); (*b*) nonspecific inhibition of both phototropism and geotropism (KCN); and (*c*) specific inhibition of phototropism (KI, NaN_3, and phenylacetic acid). Simultaneous irradiation of coleoptiles with phototropically inert light in addition to the phototropically active blue light also results in an inhibition of phototropism. Since azide, iodide, and phenylacetic acid are known to interact with flavins while a simultaneous irradiation with a phototropically inert light may depopulate the first triplet state of flavins, these data support the hypothesis that the photoreceptor pigment for phototropism in corn is a flavin.

The identification of the photoreceptor pigment controlling phototropism has in general not progressed beyond a description of action spectra for the physiological response. Although action spectra should provide, within limits, a reasonable representation of the absorption spectrum of the photoreceptor pigment, an action spectrum is insufficient for distinguishing between potential photoreceptor pigments with very similar absorption spectra (1, 3, 13). Moreover, the absorption spectrum for the photoreceptor pigment may depend strongly on its environment, but this environment is an unknown as long as the identity of the photoreceptor pigment itself is unknown.

Because of these inadequacies of action spectra as the major criterion for the photoreceptor pigment, we have searched for other criteria to identify the photoreceptor pigment for phototropism. We report here the results of experiments on the effects of potential inhibitors on phototropism and geotropism, pursuing the observation of Hart and Filner (6) that phenylacetate and potassium iodide, which complex with flavins, inhibit some responses by plants to blue light, and following their suggestion that inhibition of geotropism be used as a test for the specificity of any inhibitor of phototropism.

MATERIALS AND METHODS

Corn seedlings (*Zea mays*, hybrid MS WFg × Bear 38, Lot No. 4244 from Bear Hybrid Corn Co., Decatur, Ill.) were grown in complete darkness at 22 ± 1 C for 6 days in open trays on Kimpak germinating paper dampened with distilled H_2O. The trays were placed in large closed black boxes to keep humidity constant and the boxes were kept in a constant temperature, controlled humidity, darkroom. Only that 20% of the seedlings with straight shoots were used. The seedlings were prepared for the experiments under dim (<1 $\mu w \cdot cm^{-2}$) 540 nm (10 nm half-bandwidth) safelight. Each seedling was placed in a 50-ml test tube with the root system in a bathing solution and with 3 cm of the shoot projecting through a hole in a cork stopper inserted in the opening of the test tube. The seedlings were incubated with the roots immersed in a solution of the potential inhibitor at pH 7 for 1 hr before application of the test stimulus (light or gravity). All experiments were conducted at 22 ± 1 C.

To test for geotropism, seedlings were turned horizontal for 1 hr. The geotropic response was then permitted to develop in the vertical position for 4 hr. Phototropism was induced for 5 hr with 2 $\mu w \cdot cm^{-2}$ blue (440 nm) light from a 40 w Unitron incandescent lamp through an interference filter with a 10 nm half-bandwidth. The phototropic response to this intensity of light probably falls into the category of the second positive curvature (21). Following each experiment, the curvature was measured for each shoot, and the mean value of curvature and the standard error of the mean were calculated for the 10 to 40 seedlings for each condition. To minimize the effect of daily variations in the seedlings on the curvature data, all data were related on a percentage basis to a control group of seedlings incubated in distilled H_2O. The conditions chosen for geotropism and phototropism gave curvatures of 35 to 40° in all experiments for the seedlings incubated in the control buffer without inhibitors added. Four hr incubation in the vertical position following the 1-hr geotropic stimulation gave a maximum coleoptile curvature.

In the case of simultaneous irradiations with two wavelengths, the control group of seedlings was irradiated only with 2 $\mu w \cdot cm^{-2}$ blue light (440 nm); the test group was irradiated with the blue light and at the same time with an additional light of variable wavelength at 200 $\mu w \cdot cm^{-2}$ orthogonal to the blue light. Care was taken to assure that the test seedlings never received blue light without an irradiation with the second variable wavelength light at the same time. The variable wavelength light was defined by interference filters of 10 nm half-bandwidth with maximum transmission from 540 to 760 nm in conjunction with a Corning filter c.s. No. 3-69 to eliminate the blue light contamination from the second source. Bending by the coleoptiles was exclusively toward the blue light.

Light intensities were measured using a model 68 Kettering radiometer (Laboratory Data Control, Riviera Beach, Fla.).

[1] This work was initiated by W. S. in the laboratory of W. L. Butler at the University of California at San Diego supported in part by National Institutes of Health Grant GM-20648 to W. L. B. and continued at Michigan State University supported by U.S. Energy Research and Development Administration Contract EY-76-C-02-1338 and Deutsche Forschungsgemeinschaft grant SCH-383 to W. S.

[2] Present address: University of Konstanz, Fachbereich Biologie, D-775, Konstanz, Federal Republic of Germany.

[3] Present address: Department of Botany, University of Aberdeen, Aberdeen, U.K.

[4] To whom reprint requests should be addressed.

RESULTS

Potential Chemical Inhibitors. Potassium cyanide at concentrations from 10^{-4} to 10^{-2} M in the bathing solution surrounding the roots inhibits phototropism and geotropism to the same extent (Fig. 1D). Potassium chloride at concentrations from 10^{-3} to 10^{-1} M exhibits no significant inhibitory effect on either phototropism or geotropism (Fig. 1A). Sodium azide at 10^{-2} M inhibits both phototropism and geotropism almost completely but at concentrations less than 10^{-2} M, the inhibition of phototropism exhibits specificity, phototropism being inhibited significantly more than geotropism (Fig. 1C). The inhibitory effect of azide on geotropism is not changed by 200 $\mu w \cdot cm^{-2}$ diffuse (nondirectional) white light, showing that the inhibitory effect of azide is not light-dependent. Potassium iodide also preferentially inhibits phototropism (Fig. 1B). The preferential inhibition of phototropism by KI and the absence of such inhibition by KCl (Fig. 1B) supports the conclusion that the iodide ion is the inhibitor of phototropism and that the inhibition is not a general osmotic effect. Phenylacetic acid preferentially inhibits phototropism at 10^{-1} M and less (Fig. 1E). This specificity is not seen at 10^{-1} M when the geotropism control is tested under 200 $\mu w \cdot cm^{-2}$ diffuse white light.

Photoinhibition. When coleoptiles are exposed for 5 hr to blue (440 nm) light at 2 $\mu w \cdot cm^{-2}$ in the presence of an additional, phototropically inert light at 200 $\mu w \cdot cm^{-2}$, the observed phototropic bending is less than that observed in blue light alone. This photoinhibition of phototropic bending was measured for different wavelengths of light from 540 to 760 nm. The results (Fig. 2) show a broad region of photoinhibition over virtually the entire region studied with a maximum between 600 and 700 nm.

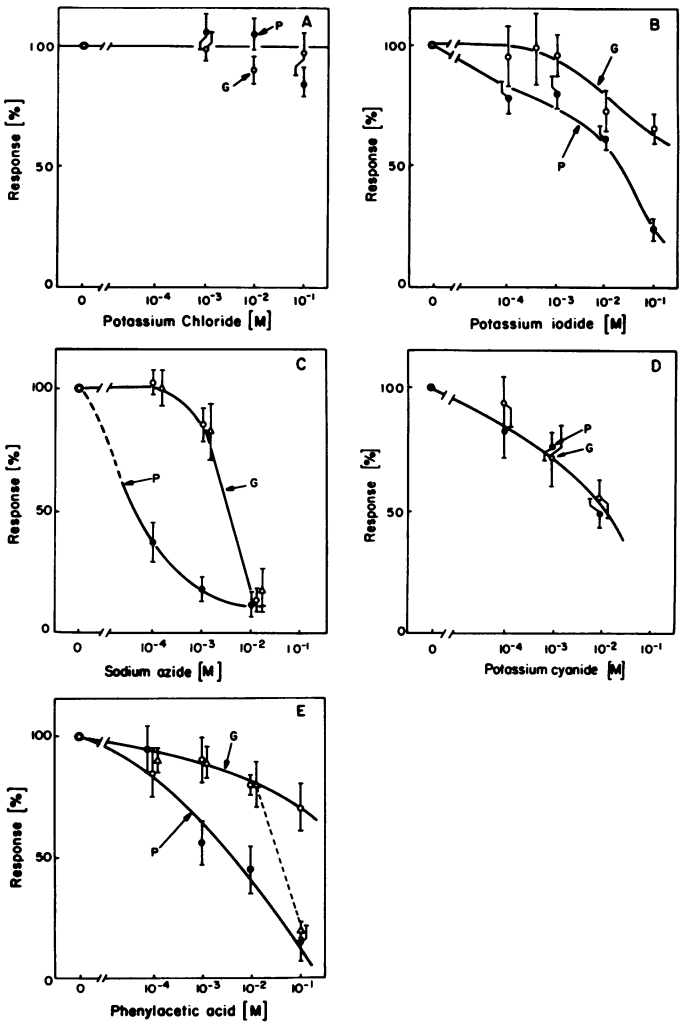

FIG. 1. Phototropic and geotropic bending in the presence of various potential inhibitors. Phototropic bending (●——●) relative to control seedlings in distilled H_2O was measured following 5 hr illumination by 2 $\mu w \cdot cm^{-2}$ blue light (440 nm). Geotropic bending relative to control seedlings in distilled H_2O was measured 4 hr after presentation of a 1-hr geotropic stimulus given in darkness (○——○) or under diffuse white light (△——△). Vertical bars represent the standard error of the mean. Potential inhibitors were: (A) KCl; (B) KI; (C) NaN_3; (D) KCN; (E) phenylacetic acid. Shoot curvature of control seedlings averaged 35 to 40° in all cases. Each point in (A) represents data from 10 test seedlings compared with 10 control seedlings. Each point in (B) through (E) represents composite data from two to four independent experiments each comparing 10 test seedlings with 10 control seedlings.

DISCUSSION

We have assumed that geotropism and phototropism in corn coleoptiles share most of their metabolic pathways, differing primarily at the sensory input. Recent evidence indicates that this assumption would probably not be correct if the research material were roots, or perhaps hypocotyls, but, in the case of coleoptiles, the assumption seems still to be valid (5, 9, 20). Thus, we have used geotropism as a control for the specificity of any potential inhibitor of phototropism. Five potential inhibitors were tested and it was found that they fall into three classes, namely: no inhibition (KCl), inhibition without observable specificity (KCN), and inhibition with specificity for phototropism (KI, NaN_3, and phenylacetic acid). This difference in the activity of different inhibitors in relation to phototropism provides a justification for using geotropism as a control for the specificity of a potential inhibitor of phototropism. Although azide is an inhibitor of metabolism in general, *e.g.* of oxidative phosphorylation (14), we have found it to inhibit phototropism more than geotropism. One possible explanation for this selective inhibition of phototropism by azide is that phototropism is more sensitive to an inhibition of oxidative phosphorylation than is geotropism. However, this explanation would imply that KCN, another inhibitor of oxidative phosphorylation, should also specifically inhibit phototropism and such a specificity is not observed. An alternative hypothesis is that azide is produc-

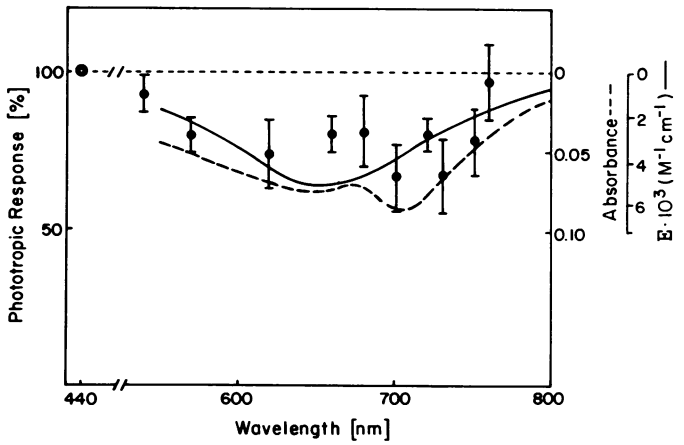

FIG. 2. Photoinhibition of blue light-induced phototropism. Each point represents mean curvature of 40 coleoptiles of seedlings irradiated with 200 $\mu w \cdot cm^{-2}$ inhibiting light orthogonal to a blue (440 nm) light (2 $\mu w \cdot cm^{-2}$) relative to a control group of 40 seedlings exposed to blue light alone. Vertical bars represent the standard error of the mean. Solid line represents absorption for the first triplet state of lumiflavin from Tegnér and Holström (18); heavy dashed line represents absorption of the first triplet state of FAD from McKellar *et al.* (10). Light dashed line represents phototropism in control seedlings exposed to blue light alone. Curvature of control seedling coleoptiles averaged 35 to 40° in all cases.

ing a strong inhibition of phototropism through a specific effect at or near to the sensory transducer itself, in addition to a weaker, less specific inhibition of some step common to the pathways of both phototropism and geotropism. Similarly, because of the apparent specificity of the action of KI and phenylacetic acid, we suggest that over some concentration range, these compounds exert a specific inhibition at or near the photosensory transduction mechanism, apart from a more general inhibition of metabolism exerted at higher concentrations.

We cannot exclude the possibility that azide, iodide, and phenylacetic acid exert a specific inhibition of phototropic bending through separate effects on different metabolic processes specific for phototropism. However, it is simpler to look for a single specific site inhibited by all three inhibitors, since these are all known to be flavin inhibitors. Iodide ions are known to be inhibitors of excitation energy transfer by various pigments including flavins (15), and an *in vivo* inhibition of a photoresponse by iodide has recently been interpreted as evidence for the involvement of the excited state of a flavin (11). Azide also has been described as an inhibitor of electron transfer involving flavins in an *in vitro* system (12). Finally, the benzyl residue of the phenylacetic acid covalently binds to the flavin nucleus of an illuminated flavin forming a phenyl derivative of the latter (7). We suggest that the specific inhibitory action of iodide, azide, and phenylacetate on phototropism is based upon their inhibition of a flavin, and that this flavin is the photoreceptor pigment itself.

Since phenylacetic acid would covalently bind to any available cellular flavin in light, and since only one flavin (the photoreceptor pigment) is postulated as specific to phototropism, the specific effect of phenylacetic acid must result from a greater accessibility of the hypothetical flavin photoreceptor pigment to phenylacetic acid. There is evidence in *Phycomyces* that the photoreceptor pigment for phototropism is localized on the plasma membrane (8). A flavin so localized would be expected to be more accessible to an inhibitor diffusing into the cell from without than would a flavin localized, for example, within the mitochondria. The specificity with which phenylacetic acid inhibits phototropism, and the fact that phenylacetic acid covalently binds to an illuminated flavin offer the potential for specific photoaffinity labeling of the photoreceptor pigment controlling phototropism. Phenylacetic acid should be replaced for such photoaffinity labeling experiments with another compound capable of forming a phenyl derivative of an illuminated flavin (7), but with one lacking the auxin-like activity of phenylacetic acid (19).

If the photoreceptor pigment is a flavin, and if the triplet state of the flavin is the active species (17) produced following excitation by blue light, then one may be able to inhibit phototropism by using phototropically inert light to depopulate the triplet state. Such light, absorbed by the active first triplet state, would excite this state and thereby shift the population density to higher triplet states; this in turn would result in a reduction in concentration of the active species and therefore decrease phototropism. The crude action spectrum presented here (Fig. 2) for the photoinhibition of phototropic bending is similar to the absorption spectra in the literature for the first triplet state of flavins (10, 18). Song and Moore (16) have discussed the reason for considering the photobiological activity of the triplet state of a carotenoid as unlikely.

Chon and Briggs (2) have shown a phytochrome-phototropism interaction, irradiation with red light inhibiting subsequent blue light-induced phototropism in corn and far red light reversing this effect. It should be noted that in the experiments of Chon and Briggs (2), the inhibiting irradiations were given before the first positive phototropic curvature was induced. The experiments presented here employed simultaneous irradiations, and the second positive curvature was measured. We cannot eliminate the possibility that photoactive pigments such as phytochrome or Chl are responsible for or play some role in this inhibition of phototropism by concurrent red light irradiation. The action spectrum presented here, while similar to the absorption spectrum for the first triplet state, is difficult to reconcile with known absorption spectra for either phytochrome or for Chl. A recent experiment by Delbrück *et al.* (4) with *Phycomyces* supports the view that the active species for phototropism is the first triplet state. This experiment involved the use of a high intensity light from a tunable dye laser to induce the "forbidden" electronic ground state-to-first triplet state transition resulting in a phototropic response. It may be possible to separate the two possible red light inhibitions of phototropism (mediated by phytochrome, or mediated via a depopulation of the first triplet state) through the use of an organism such as *Phycomyces* which lacks phytochrome and the photosynthetic pigments. The dose (2 $\mu w \cdot cm^{-2}$ for 5 hr) of blue light used would clearly elicit the second positive curvature (21). These conditions were chosen to maximize the time for interactions between the irradiated photoreceptor pigment and the potential inhibitor, and to minimize any dark period during which inhibitor-irradiated pigment interactions would not occur while inhibitor-tissue interactions would continue. Additional information should now be obtained through the measurement of phototropic dosage response curves under conditions of simultaneous red light irradiation. Such curves would permit an evaluation of the effects of simultaneous red light on the first and second positive curvatures and could be compared with similar curves of Zimmerman and Briggs (21) under conditions of prior red light irradiation.

LITERATURE CITED

1. Checcucci A, G Colombetti, R Ferrara, F Lenci 1976 Action spectra for photoaccumulation of green and colorless *Euglena*: evidence for identification of receptor pigments. Photochem Photobiol 23: 51-54
2. Chon HP, WR Briggs 1966 Effect of red light on the phototropic sensitivity of corn coleoptiles. Plant Physiol 41: 1715-1724
3. De Fabo, EC, RW Harding, W Shropshire Jr 1976 Action spectrum between 260 and 600 nanometers for the photoinduction of carotenoid biosynthesis in *Neurospora crassa*. Plant Physiol 57: 440-445
4. Delbrück M, A Katzir, D Presti 1976 Response of *Phycomyces* indicating optical excitation of the lowest triplet state of riboflavin. Proc Nat Acad Sci USA 73: 1969-1973
5. Digby S, RD Firn 1976 A critical assessment of the Cholodny-Went theory of shoot geotropism. Curr Adv Plant Sci 8: 953-960
6. Hart J, P Filner 1967 Physiological effect of photoreduction of flavin. Plant Research '67. Ann Rept MSU/AEC Plant Res Lab, E Lansing Michigan, pp 28-30
7. Hemmerich P, V Massey, G Weber 1967 Photo-induced benzyl substitution of flavins by phenylacetate: a possible model for flavoprotein catalysis. Nature 213: 728-730
8. Jesaitis AJ 1974 Linear dichroism and orientation of the *Phycomyces* photopigment. J Gen Physiol 63: 1-21
9. Juniper, BE 1976 Geotropism. Annu Rev Plant Physiol 27: 385-406
10. McKellar JF, GO Phillips, A Checcucci, F Lenci 1976 Excited states of flavins in photoreception processes. *In* JB Birks, ed, Excited States of Biological Molecules. John Wiley & Sons, New York, pp 92-105
11. Mikolajczyk E, B Diehn 1975 The effect of potassium iodide on photophobic responses in *Euglena*: evidence for two photoreceptor pigments. Photochem Photobiol 22: 269-271
12. Schmidt W, WL Butler 1976 Flavin-mediated photoreactions in artificial systems: a possible model for the blue-light photoreceptor pigment in living systems. Photochem Photobiol 24: 71-75
13. Shropshire W Jr 1972 Action spectroscopy. *In* K Mitrakos, W Shropshire Jr, eds, Phytochrome. Academic Press, New York, pp 161-181
14. Slater EC 1967 Application of inhibitors and uncouplers for a study of oxidative phosphorylation. Methods Enzymol 10: 48-57
15. Song PS, TA Moore 1968 Mechanism of the photodephosphorylation of menadiol diphosphate. A model for bioquantum conversion. J Am Chem Soc 90: 6507-6514
16. Song PS, TA Moore 1974 On the photoreceptor pigment for phototropism and phototaxis: is a carotenoid the most likely candidate? Photochem Photobiol 19: 435-441
17. Song PS, TA Moore, M Sun 1972 Excited states of some plant pigments. *In* CO Chichester, ed, The Chemistry of Plant Pigments. Academic Press, New York, pp 33-74
18. Tegnér L, B Holström 1966 A fast reacting intermediate in flash excited flavin solution. Photochem Photobiol 5: 223-226
19. Wightman F, BS Rantham 1973 Evidence for the biosynthesis and natural occurrence of the auxin, phenylacetic acid, in shoots of higher plants. *In* Plant Growth Substances 1973. Hirokawa Publishing Co Inc, Tokyo, pp 15-27
20. Wilkins MB 1966 Geotropism. Annu Rev Plant Physiol 17: 379-408
21. Zimmerman KB, WR Briggs 1963 Phototropic dosage response curves for oat coleoptiles. Plant Physiol 38: 248-253