Azido Auxins

PHOTOLYSIS IN SOLUTION AND COVALENT BINDING TO SOYBEAN

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ABSTRACT

The potential of three auxin analogs, 4-, 5-, and 6-azidoindole-3-acetic (4-N3IAA, 5-N3IAA, and 6-N3IAA), as photoaffinity labeling agents for the detection and isolation of auxin receptors was assessed by irradiating these compounds at 365 nm on TLC plates, in solution, and in contact with soybean (Glycine max L. Merr. var. Wayne) hypocotyl. Photolysis on TLC plates produces immobile spots, indicating extremely polar covalent binding of the photoproducts to the plates. On irradiation in buffer or in buffer containing sucrose, all three compounds decompose at rates that are first order in N3IAA to give fluorescent solutions. Photolysis through a Pyrex filter is slower than that through quartz, but the filter prevents tissue damage and allows a given dose of irradiation to photolyze all three N3IAAs to the same extent. The effects of photolysis of these compounds in vivo were evaluated with a straight growth assay using etiolated soybean hypocotyl segments. According to this assay, the photoproducts of the N3IAAs possess little auxin activity. Irradiation of soybean hypocotyl tissue after 1-hour exposure to 4-N3IAA in the dark causes the tissue to grow during 12 hours as much tissue that is continuously exposed to 4-N3IAA in the dark for this period, suggesting that, on photolysis, this auxin analog binds irreversibly to an auxin-sensitive site. Although the fluorescence intensity of the photolyzed N3IAAs is weak enough to require another method of detecting the bound analog under physiological conditions, the evidence for covalent binding of the N3IAAs on photolysis implies that these compounds will be satisfactory photoaffinity labeling agents.

Earlier, we reported the synthesis of 4-, 5-, and 6-azido-3-indoleacetic acid and showed that, in the dark, these compounds are physiologically indistinguishable from IAA in every system tested (14). As a continuation of the assessment of their usefulness as fluorescent photoaffinity labeling agents, we now describe the photolysis of these compounds on TLC plates, in solution, and in vivo. In particular, we discuss the conditions required for photolysis of the N3IAAs, the fluorescence and biological activity of the photoproducts, and the ability of the N3IAAs to bind covalently on photolysis. We also describe a sustained response of soybean hypocotyl segments to one of the N3IAAs under conditions intended to produce covalent binding of this IAA analog to a physiologically significant site. In the sequel (10), we provide quantitative binding data for the N3IAAs in maize. In a future paper, we will report experiments using radiolabeled N3IAAs to locate auxin-binding proteins.

MATERIALS AND METHODS

Plant Material. Glycine max L. Merr. var. Wayne was sown onto moist vermiculite and harvested after 3.5 d. Unless otherwise noted, all operations involving seed or plant tissue were done in the dark or under green light at 27°C and 85% RH.

Chemicals. High quality, white, crystalline IAA (melting point = 167-169°C) was purchased from Sigma and stored in a brown vial at −5°C. The preparation of 4-, 5-, and 6-N3IAA has been described (14). These compounds were stored in brown, foil-wrapped vials at 3°C. Unless noted otherwise, all manipulations of N3IAAs were performed in the dark or under red or green light. Samples of IAA and the N3IAAs for quantitative spectroscopy and for photolysis in solution were weighed by Josef Nemeth and his staff at the University of Illinois. Buffer was 5 mm K-phosphate, pH 6.0, and sucrose-buffer was 30 mm sucrose and 5 mm K-phosphate, pH 6.0. Stocks and solutions described below were stored at 3°C; those containing N3IAAs were wrapped in aluminum foil.

Equipment. Electronic absorption spectra were recorded on a Beckman Acta MVI from 350 to 200 nm, unless stated otherwise. Except where noted, samples were in 1-cm path length silica cells with water as reference and blank. Fluorescence emission spectra were obtained on a Spex Fluorolog attached to a circulating Lauda K-2/RD constant temperature bath. Samples were allowed to equilibrate at 20°C for 10 min before fluorescence emission spectra were recorded. For photolysis in solution and on TLC plates, the UV source was a Mineralight lamp from UV Products (San Gabriel, CA) containing a 15-cm 6-w bulb (365-nm peak maximum). For photolysis involving plant tissue, the UV source was a desk lamp containing a 46-cm General Electric F15T8-BL 15-w bulb (365-nm peak maximum). The intensity of the light delivered was controlled by adjusting the distance between the sample and the lamp. The correlation between distance and intensity was measured with a Blak-Ray UV light meter (model J-227) attached to a long wave sensor cell (model J-222), both from UV Products. The vessel for irradiation of solutions involving the use of plant tissue was a rectangular Plexiglas box divided into eight compartments. After segments (where appropriate) and solutions were distributed among the compartments, the box was covered with a double layer of Saran Wrap to reduce evaporation and with an inverted Pyrex Petri dish to filter out irradiation below 300 nm. The box was clamped to a shaker with the plane of the top of the box parallel to the bottom of the lamp housing and centered below it in such a...
manner that the box moved with the shaker parallel to the long axis of the lamp. The distance between the box and the lamp was adjusted so that the intensity of irradiation delivered to the top surface of the solutions in the absence of the Pyrex filter was 250 \( \mu W/cm^2 \) (cf. 2 mw/cm\(^2\) in Ref. 10).

**Photolysis on TLC Plates.** IAA, 4-, 5-, and 6-N\(_3\)IAA in methanol were spotted separately on the right and left halves of three types of TLC plates, giving two identical sets of spots on each plate. Plates (all with fluorescent indicator) and developing solvents were as follows: silica gel (Brinkmann), ethyl acetate:2-propanol:water, 65:25:10 (v/v); alumina (Eastman), 1-butanol:water:acetic acid, 4:1:1 (v/v); and cellulose (Eastman), water. The left half of the plate was covered with cardboard and the right half was irradiated for 5 min at 200 \( \mu W/cm^2 \). After irradiation, the plate was developed and the solvent was removed with an air blower at room temperature. Visualization was under 254 and 365 nm light. Each experiment was performed at least twice.

**Spectroscopy.** Solutions approximately 50 \( \mu W \) in IAA, 4-, 5-, or 6-N\(_3\)IAA (in buffer or in sucrose-buffer) were prepared quantitatively from accurately weighed samples. The insolvility of these compounds required that they first be dissolved in the calculated amount of aqueous KOH. Solutions were placed in matched, 1 x 1 cm quartz cuvettes, and electronic absorption spectra were recorded from 600 to 200 nm. Fluorescence emission spectra were recorded from 300 to 600 nm with an excitation wavelength of 292 nm and a slit width of 10 nm. References and blanks were either buffer or sucrose-buffer, as required.

**Photolysis in Solution.** Fresh solutions (see “Spectroscopy”) were placed in tightly stoppered, matched, 1 x 1 cm quartz cuvettes. Electronic absorption and fluorescence emission spectra were recorded under conditions described above. The cuvettes were placed on their sides on a white piece of paper and irradiated at room temperature for approximately 2 h with light having an intensity of 140 \( \mu W/cm^2 \) at the top rectangular surface of the cuvette. These reactions were monitored by periodically interrupting irradiation to record electronic absorption and fluorescence emission spectra. The solutions were allowed to stand overnight in the dark at room temperature, after which electronic absorption and fluorescence emission spectra were recorded again. Photolysis was continued for another 2 h, followed by electronic absorption and fluorescence emission spectroscopy. The \( \phi \) of the photolyzed solution was 6.00 ± 0.03 in all cases. Each photolysis was performed in duplicate.

**Kinetic Analysis.** Photolyses were analyzed by plotting the logarithm of the fraction of starting material remaining, \( x \), **versus** time, where \( x = (A - A_t)/(A_0 - A_t) \). \( A_0 \) is the initial absorbance, \( A \) is the absorbance after an indicated time of photolysis, and \( A_t \) is the absorbance after 2 h (near completion). The resulting straight lines fit the equation \( -2.303 \log \frac{x}{k} = kt \). First order rate constants, \( k \), were determined graphically, from the negative slopes of the lines, and by the method of least squares. In most cases, these values were checked by computation from data taken at more than one wavelength. From the rate constants, half-lives, \( t_{1/2} \), were calculated from the equation \( t_{1/2} = 0.693/k \). Where possible, photolyses were also analyzed by plotting the logarithm of the fluorescence intensity, \( I \), **versus** time, where \( I \) is the fluorescence intensity after an indicated time of photolysis. The resulting straight lines fit the equation \( 2.303 \log I = kt \). First order rate constants, \( k \), were determined graphically.

**Bioassay.** The effect of each treatment described below was evaluated by a straight growth assay using etiolated soybean hypocotyl tissue. Stem segments 10 mm long were excised from the region beginning 3 mm below the hypocotyl hook, just at the point where the diameter of the stem ceased widening. Segments were preincubated in sucrose-buffer (50 segments/25 ml) for 1 h in a shaker to deplete endogenous auxin and were rinsed three times with 100-ml portions of water. At the beginning of each experiment, 10 preincubated, washed tissue segments were measured to give an average initial length. At the end of each experiment, 10 treated tissue segments were immediately placed on ice, drained, and measured to give an average final length.

**Rate of Photolysis through a Pyrex Filter.** Stocks of IAA, 4-, 5-, and 6-N\(_3\)IAA (1 mM) were prepared by dissolving these compounds in a few drops of 1 N KOH, diluting with water to the approximate final volume, adjusting to pH 6.0 with 0.1 N HCl, and diluting with water to the exact final volume. IAA stocks were discarded after 1 week, but the N\(_3\)IAA stocks were discarded only if they became turbid or sowed other evidence of growth of mold. With these safeguards, no decomposition of IAA or N\(_3\)IAA stocks was detectable by TLC, UV, or bioassay. Aliquots (4 ml) of 10 \( \mu W \) IAA, 4-, 5-, and 6-N\(_3\)IAA in sucrose-buffer prepared from these stocks were distributed in the Plexiglas box after an electronic absorption spectrum of each solution was recorded, and the solutions were shaken and irradiated as described above. Irradiation was periodically interrupted to remove the contents of one compartment, and an electronic absorption spectrum of this partially photolyzed solution was recorded. Irradiation was continued until the spectra of two consecutive aliquots were superimposable. This procedure was performed twice for each compound.

**Auxin Activity of Photoproducts.** This procedure, designated photoproduct treatment, is summarized in Scheme 1. Three 4-

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**Scheme 1**

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**Table**

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ml samples of 10 \( \mu W \) IAA, 4-, 5-, or 6-N\(_3\)IAA in sucrose-buffer (see “Rate of Photolysis through a Pyrex Filter”) or of sucrose-buffer only were irradiated in the Plexiglas box as described above, for various periods up to 24 h. One 4-ml samples was used to record an electronic absorption spectrum. The other two were placed in 25-ml Erlenmeyer flasks each containing 10 segments of preincubated, washed tissue. The flasks were covered loosely, and the segments were incubated in a shaker for 12 h. Experiments involving irradiation for 50 min, 5.5 h, and 24 h were performed only once; experiments involving no irradiation and irradiation for 8 h were performed three to six times.

**Photolysis In Vivo.** These procedures, designated constant, dark, and light treatments, are summarized in Scheme 1. Ali-
RESULTS

Photolysis on TLC Plates. Brief photolysis of 4-, 5-, and 6-N3IAA on three types of TLC plates produced spots that did not move on development, despite the high polarity of the chosen eluants (Fig. 1). In each case, intact N3IAAs were also detectable, as expected for partial photolysis. Although the number, mobility, fluorescence, and stability of the photoproducts depended upon the isomer and the plate type, photolysis under a given set of conditions was reproducible. For IAA itself, no photolysis and no tight binding to the plate were observed.

Spectroscopy. Electronic absorption and fluorescence emission spectra of IAA and the N3IAAs in buffer are summarized in Table I. Spectra of all four compounds agree with those previously obtained in water or in 95% ethanol (3, 7, 14). The N3IAAs are nonfluorescent, as expected. In all cases, spectra were unaffected by the addition of sucrose. Because the fluorescence intensity of many indoles is temperature-dependent (20) and because azides are photolabile, fluorescence emission spectra of these compounds were recorded at constant temperature and rapid scan rates. Neither precaution, however, was found to be necessary for obtaining reproducible electronic absorption spectra.

Photolysis in Solution. IAA, 4-, 5-, and 6-N3IAA were irradiated with 365 nm light in buffer and in sucrose-buffer. The pH remained constant throughout photolysis. Decomposition was monitored by electronic absorption and fluorescence emission spectroscopy. In both solvents, after 2 h of irradiation, the electronic absorption and fluorescence emission spectra of IAA revealed slight decomposition, the extent of photolysis being somewhat greater in the absence of sucrose. By contrast, irradiation of the three N3IAAs was accompanied by pronounced changes in both types of spectra.

Most of the changes in the electronic absorption spectra occurred within the first 2 h of irradiation. Further minor changes occurred on prolonged irradiation or on storage in the dark for the 4- and 6- but not for the 5-N3IAA. Analysis of the electronic absorption spectra as described in “Materials and Methods” showed that all photolyses were first order in N3IAA. The photolytic half-lives for these reactions, displayed in Table I, show that, in buffer, the 4-N3IAA decomposes more rapidly than 6-N3IAA and that 5-N3IAA is slowest to disappear. Neither the rates of decomposition nor the appearance of the final spectra were affected by the presence of sucrose.

Changes in the fluorescence emission spectra were more complex. As shown in Table I, each N3IAA produced one or more fluorescence emission maxima on photolysis. The fluorescence intensity of most of these peaks continued to undergo significant changes on prolonged irradiation or on storage in the dark. In general, the rates of increase of the intensities of these peaks did not agree with the rates of disappearance of the corresponding N3IAA calculated from changes in the electronic absorption spectra. Except for 5-N3IAA, the fluorescence intensity of the photolyzed solutions was weak compared with that for equivalent

Table 1. Electronic Absorption and Fluorescence Emission Spectra of IAA, the N3IAAs, and Their Photoproducts in Buffer

<table>
<thead>
<tr>
<th>Compound</th>
<th>Electronic Absorption (λmax)</th>
<th>Fluorescence Emission (λmax)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(nm (log ε))</td>
<td>(nm)</td>
</tr>
<tr>
<td>Starting materials</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IAA</td>
<td>219 (4.63), 271 (3.83), 278 (3.86), 287 (3.79)</td>
<td>363</td>
</tr>
<tr>
<td>4-N3IAA</td>
<td>226 (4.43), 297 (3.98)</td>
<td></td>
</tr>
<tr>
<td>5-N3IAA</td>
<td>244 (4.41), 300 (3.60), 313 (3.41)</td>
<td>b</td>
</tr>
<tr>
<td>6-N3IAA</td>
<td>215 (4.26), 246 (4.34), 288 (3.99)</td>
<td>b</td>
</tr>
<tr>
<td>Photoproducts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IAA</td>
<td>217 (4.59), 253 (3.75), 272 (3.81), 279 (3.83), 288 (3.77)</td>
<td>363 (4a)</td>
</tr>
<tr>
<td>4-N3IAA</td>
<td>226 (4.25), 280–301 (3.87)</td>
<td>363, 450, 585 a</td>
</tr>
<tr>
<td>5-N3IAA</td>
<td>242 (4.23), 288 (3.84)</td>
<td>375</td>
</tr>
<tr>
<td>6-N3IAA</td>
<td>215 (4.17), 246 (4.01), 288 (3.80)</td>
<td>b</td>
</tr>
</tbody>
</table>

* Excitation wavelength was 292 nm, slit width was 10 nm, temperature was 20°C.

a These compounds are nonfluorescent.
b Solutions were photolyzed with 365-nm light through quartz as described in “Materials and Methods.”
c Intensity slightly reduced.
d Plateau.
e Shoulder.
concentrations of IAA.

**Effect of a Pyrex Filter.** A Pyrex filter was used for photolysis *in vivo* to prevent irradiation damage of plant tissue. Preliminary experiments showed that the ability of unprotected tissue to respond to IAA is greatly reduced by irradiation prior to the addition of IAA. A Pyrex filter, which absorbs light below 300 nm, prevents such damage, whereas Saran Wrap, which is transparent below 300 nm, provides no protection.

Results of photolysis of the N₃IAAs through a Pyrex filter under conditions used *in vivo* were similar to those obtained on photolysis through quartz as described above. Photodecomposition on irradiation through Pyrex was first order, as was the case on irradiation through quartz. Electronic absorption spectra of completely photolyzed solutions differed only slightly from those obtained on photolysis through quartz. Rates of photolysis on irradiation through Pyrex were much slower than on irradiation through quartz, however, as shown in Table II, and were similar for all three N₃IAAs, in contrast to the different rates of photolysis observed for each N₁IAA on photolysis through quartz.

**Auxin Activity of Photoproducts.** The auxin activity of the photoproduc.ts of the N₁IAAs was assessed by photolyzing growth media through a Pyrex filter for various lengths of time before tissue segments were added, as shown in Scheme 1, photoproduct treatment. Sucrose-buffer and IAA in sucrose-buffer served as controls. As shown in Figures 2 and 3, solutions of N₁IAAs that have been photolyzed for 8 h display very little auxin activity in the soybean assay. From the measured half-lives of these compounds on irradiation through a Pyrex filter, the concentration of N₁IAA remaining after 8 h was calculated to be less than 10 nm in agreement with the virtually complete decomposition revealed by the electronic absorption spectra of these solutions. Moreover, solutions of N₁IAAs irradiated for 24 h show no further diminution of auxin activity and little further change in electronic absorption spectra. Thus, the slight auxin activity observed after 8 h of photolysis is unlikely to be attributable to any residual N₁IAA. More probably, the small amount of growth observed is due to weak auxin activity of the photoproducts themselves. Solutions containing IAA irradiated for 8 h display a slight depression in auxin activity, in agreement with the partial photolysis of IAA observed earlier (see "Photolysis in Solution") and with the decomposition of IAA by light reported by others under different conditions (1, 12, 18, 22). As expected, irradiation of sucrose-buffer before addition of tissue segments had no effect on the minimal growth observed in this medium.

**Photolysis in Vivo.** The ability of the N₁IAAs to bind covalently upon photolysis *in vivo* was assessed by growing segments of soybean hypocotyl under three sets of conditions. These treatments, designated constant, dark, and light, are summarized in Scheme 1 and the results are displayed in Figure 3. For each treatment, sucrose-buffer and IAA in sucrose-buffer were included as controls. In all three treatments, segments in which endogenous auxin had been depleted were incubated for 1 h in solutions containing no auxin, IAA, or N₁IAA. The purpose of this loading step was to allow IAA or N₁IAA to permeate the tissue and to occupy auxin-sensitive sites. At this point, the treatments diverged.

In the constant treatment, tissue was grown in the dark for an additional 11 h in solutions containing no auxin, IAA, or N₁IAA. Under these conditions, all three N₁IAAs caused growth comparable to that of IAA, in agreement with our previous report (14). The low growth observed under these conditions in sucrose-buffer implies that endogenous auxins were not being regenerated in significant amounts during the course of the experiments.

In the dark treatment, after the initial 1-h exposure to solutions containing no auxin, IAA, or N₁IAA, tissue was transferred to fresh sucrose-buffer in which it was grown for an additional 11 h. Again, all three N₁IAAs behaved like IAA. As might be expected considering the shorter exposure to IAA or N₁IAA, tissue grew less than when hormone was present for the entire growing period. The possibilities were investigated separately that the differences in growth observed between the dark and constant treatments resulted from the additional handling of the tissue or the extra washing and change in solution associated with the dark treatment. No tactile response was observed, within experimental error. The effect of the extra washing and change in solution was small, accounting for the differences in growth in sucrose-buffer between dark and constant treatments, but not for the differences in IAA or in N₁IAA.

In the light treatment, after tissue had been exposed to N₁IAA during the loading phase, it was transferred to fresh sucrose-
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Fig. 3. Growth of soybean hypocotyl segments according to treatments delineated in Scheme 1. Solutions contained 10 μM IAA (4); 4-N3IAA (6); 5-N3IAA (2); or 6-N3IAA (2) (all in sucrose-buffer) or sucrose-buffer only, 8. Growth is expressed as final length minus initial length. Each bar represents the average of three to six experiments.

buffer, as in the dark treatment. In the light treatment, however, the tissue was irradiated for 50 min before being returned to darkness for the remainder of the growing period. In this treatment, IAA, 5-, and 6-N3IAA again behaved alike, showing significantly reduced growth, but the 4-isomer behaved quite differently, showing full auxin activity. To confirm this unusual result, we repeated the experiment 10 times (including the six times reported here), using two different seed lots, over the space of 2 years, with three different people performing the comparative experiments.

DISCUSSION

With few exceptions (16, 21), attempts to locate and isolate plant hormone receptors have relied upon equilibrium binding techniques. Such techniques suffer inherent limitations, the most serious being the lability of ligand-receptor complexes. Our investigation is based on covalent attachment of a special class of auxin analog, the N3IAAs, to auxin receptors using the technique of photoaffinity labeling.

In this technique (2, 4-6, 11, 13), a photoactive substrate analog binds reversibly to a receptor in the dark. On photolysis, the analog generates a chemically reactive species, which undergoes covalent attachment to the active site of the receptor. If diffusion of the reactive intermediate away from the active site is rapid compared with the rate of covalent attachment, binding to the receptor outside the active site, to extraneous tissue components, or to the solvent can occur. Reactive intermediates that have become bound to solvent can be removed easily by washing, as can those that have achieved stability through rearrangement or abstraction rather than through addition or insertion. Labeling outside the active site or on extraneous tissue components (nonspecific labeling) can be a serious problem, however, since these molecules cannot be washed away. At high levels, such labeling can obscure labeling inside the active site (specific labeling).

The results of photolysis of the N3IAAs on three types of TLC plates are encouraging, particularly those for cellulose, the support providing the closest analogy to plant systems. The photoproducts are fluorescent, at least when bound to alumina and cellulose, and the tight binding of most photoproducts to the TLC plates indicates extremely polar or covalent binding.

Photolysis in solution leads to several important conclusions. First, the N3IAAs are readily photolyzable in the absence of a Pyrex filter, having half-lives on the order of minutes under these conditions. Second, sucrose, a major constituent of the growth media used for photolysis in vivo has no effect on the spectra of the N3IAAs or on the course of photolysis. Third, like photolysis on TLC plates, photolysis in solution generates multiple products, some of which are unstable. Photolyzed 4-N3IAA, for example, gives three widely spaced fluorescence emission maxima, exhibiting different rates of increase and decay of fluorescence intensity, none of which agrees with the rate of disappearance of 4-N3IAA calculated from changes observed in the electronic absorption spectra. These complexities are probably not associated with the main reaction, however. If they were, changes in the electronic absorption and fluorescence emission spectra would be more closely correlated. The observed fluorescence intensities are too low to be experimentally useful in detecting ligand-bound receptors. Fortunately, however, other sensitive methods for detecting bound photoaffinity labels exist. One possibility is radioimmunoassay of the type developed by Pengelly and Meins for IAA (17). Another alternative is radiolabeling. We have already reported the preparation of 5-azido[7-3H] indole-3-acetic acid (15) and are currently synthesizing two other tritiated N3IAAs.

Our experience with photolysis in solution guided our studies of photolysis in vivo. The principal modification required in vivo was the use of a Pyrex filter. Besides protecting the tissue from the filter has two significant effects. First, it greatly reduces the rate of photodecomposition by reducing the amount of light reaching the samples, especially in the high energy portion of the spectrum. Although the intensity of irradiation is increased by using a more powerful lamp and by placing it as close as possible to the samples, photosynthetic half-lives are still inconveniently long. The 50-min irradiation chosen for photolysis in vivo corresponds to only one half-life for all three isomers, yet longer times would make irradiation consume an unreasonably large fraction of the total growing time for the bioassay. The second effect of the filter is to reduce the differences in the rates of photolysis observed for the three N3IAAs on irradiation through quartz, perhaps because the 5-isomer, which is the most resistant to photolysis through quartz, is the only one of the azido auxins to have a λ_max above the Pyrex cutoff.

In most respects, the results of photolysis in vivo suggest that the technique of photoaffinity labeling works as described earlier. The similarity of the growth caused by IAA and the N3IAAs in the constant and dark treatments implies that N3IAAs bind to IAA-sensitive sites in the dark. The reduced growth induced by limited exposure to both IAA and the N3IAAs in the dark treatment suggests that, without photolysis, this binding is noncovalent, that gradual leaching of IAA and the N3IAAs from the auxin receptors into the surrounding sucrose-buffer occurs. Conclusive evidence for the reversibility of binding in the dark could be obtained by monitoring the uptake and efflux of radiolabeled IAA and N3IAAs.

The most interesting result of the light treatment is the sus-
tained growth that occurs when tissue is loaded with 4-N3IAA before irradiation. This response can be explained in terms of the model for photoaffinity labeling presented above, if we assume that covalent binding of the substrate analog to the active site of the hormone receptor 'permanently' activates the receptor response. Alternatively, we could assume that 4-N3IAA binds covalently to an enzyme or carrier rather than to a receptor, blocking normal storage, degradation, or transport of the substrate analog in such a way that the availability of the 4-N3IAA surviving photolysis is enhanced. In either case, the sustained response suggests that covalent binding has occurred.

The reduced growth observed when tissue is exposed to 5- and 6-N3IAA and to IAA during the light treatment has several possible explanations. These are mostly evaluated when grouped according to the structures presumed to be affected by irradiation, as delineated below.

The first category involves effects of light on tissue only. Irradiation damage to the tissue itself is not responsible for reduced growth, since tissue protected by Pyrex during irradiation in the absence of any auxin shows no diminution of responsiveness to IAA added later. The possibility that IAA enhances tissue damage has not been ruled out, however.

The second category includes several effects of light on free ligands. (a) Photodestruction of IAA, possibly including conversion to an auxin inhibitor, is probably responsible for the reduced growth observed with this compound, since electronic absorption and fluorescence emission spectra of solutions irradiated in the absence of plant tissue show slight decomposition.

(b) Decomposition of the photolabile N3IAAs may be responsible for the reduced growth observed for 5- and 6-N3IAA. However, because the 50-min photolysis is insufficient to destroy all of the N3IAAs under the light flux we are using, tissue in media containing 5- and 6-N3IAA grows more than in sucrose-buffer alone.

(c) Differences between 4-N3IAA and 5- and 6-N3IAA are not the result of differential destruction of these IAA analogs, since a given dose of UV irradiation through Pyrex photolyses each isomer to the same extent.

(d) Observed growth in the presence of the N3IAAs is not due to photoproducts, since these possess little auxin activity in the soybean assay. This is true for 4-N3IAA as well as for 5- and 6-N3IAA.

The third category consists of possible effects of light on a complex between the ligands and some cellular component(s). (a) Differences in the rate of diffusion of the various N3IAAs away from the active site(s) before their conversion to reactive intermediates are probably not responsible for the differences in growth between tissue grown in the presence of 4-N3IAA and of 5- and 6-N3IAA in the light treatment, since, in the dark treatment, where diffusion occurs freely, all three N3IAA's display similar levels of activity.

(b) Differences in reactivities and lifetimes of intermediates generated on photolysis may account for the observed differences in the activities of the N3IAAs in the light treatment. Testing the validity of this hypothesis requires a better understanding of the course of photolysis from a chemical standpoint. Such a study is in progress. Related systems are already known to show different behavior on photolysis if the azido group is located on the 4-position than if it resides on the 5- or the 6-position (8, 9, 19).

(c) Subtle differences in the geometry of binding of the N3IAAs to the receptor may also explain the differences in the activity of the N3IAAs observed in the light treatment, since different binding orientations may activate or incapacitate the receptor to different degrees.

(d) As with 4-N3IAA, 5- and 6-N3IAA may bind covalently upon photolysis to an enzyme or carrier instead of to a hormone receptor, but the blockage may be less efficient for the 5- and 6-isomers, allowing the metabolism or transport of residual 5- and 6-N3IAA to proceed. This is a corollary of the earlier argument concerning the 4-N3IAA.

In summary, all proposed explanations for the reduced auxin activity of 5- and 6-N3IAA have been ruled out except partial photolysis of the ligands and the four possibilities involving covalent binding of the ligands to some auxin-sensitive site. The sustained response induced by 4-N3IAA accentuates the value of the N3IAAs as photoaffinity labeling agents. Determination of the nature and function of the substance(s) to which the 4-N3IAA may be bound, for example, is binding specifically upon photolysis requires further examination, but the evidence for covalent binding upon photolysis presented here is compelling. The following article describes a quantitative analysis of reversible binding of the N3IAAs to auxin-binding sites of maize coleoptiles in the dark and of covalent binding of 5-N3IAA to auxin-binding sites upon photolysis.

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LITERATURE CITED

1. AMAY-GUERRI, F. R. MARTINEZ-URRUTIA 1979 Fotooxidacion de acido 3-
6-azidoindolacetoico. Rev Acad Cien Exactas, Fis Nat Madr 73: 596–598
3. BRIDGES JW, RT WILLIAMS 1968 The fluorescence of indoles and aniline
4. CHOWDHRY V, FH WESTHEIMER 1979 Photoaffinity labeling of biological
systems. Ann Rev Biochem 48: 293–325
5. COOPERMAN BS 1976 Photoaffinity labeling of proteins and more complex
Plenum, New York, pp 1–4
6. CREE D 1974 Photochemical probes for biological interactions. Photochem
Photobiol 19: 459–462
7. DISSMAKERN L, JR LANG 1964 The protonation of indoles. Basisty studies. The
8. IDDON B, MW PICKERING, H SUSCHITZKY, DS TAYLOR 1975 Condensed thiophen
ring systems. XVIII. Thienoazepins and thienobenzoxazoles from 6
Soc Perkin Trans 1: 579–583
10. JONES AM, LL MELHADO, T-HD HO, NJ LEONARD 1984 Azido auxins: the
quantitative binding data in maize. Plant Physiol 74: 295–301
11. KATZENELLENBOGEN JA 1976 Affinity labeling as a technique in determining
hormone mechanisms. In G Litwack, ed, Biochemical Actions of Hormones,
vol 4, Academic Press, New York, pp 1–4
12. KLENGMÜLLER W 1960 Über die Photolyse von Heteroauxin in ultravioletten
13. KNOWLES JR 1972 Photogenerated reagents for biological receptor-site labeling.
Azc Chem Rev 5: 155–160
14. MELHADO LL, AM JONES, NJ LEONARD, LV VANDENHOEF 1981 Azido auxins:
synthesis and biological activity of fluorescent photoaffinity labeling agents.
Plant Physiol 68: 469–475
15. MELHADO LL, CJ PEARCE, M D’ALLARCO, NJ LEONARD 1982 Specifically
deuterated and tritiated auxins. Phytochemistry 21: 2879–2885
16. MOORE FH III 1979 A cytokinin-binding protein from wheat germ: isolation by
affinity chromatography and properties. Plant Physiol 64: 594–599
17. PENGELLEY W, F MINNS JR 1977 A specific radioimmunoassay for nanogram
quantities of the auxin, indole-3-acetic acid. Planta 136: 173–180
18. RAY P, G CURRY 1958 Intermediates and competing reactions of the photo-
19. SCOVEN EPV, H SUSCHITZKY, DR THOMAS, RF NEWTON 1979 Decomposition
of some substituted azidoindoles and azidohexahydrocarbazoles. J Chem
Soc Perkin Trans 1: 53–59
Press, New York, pp 106–107
22. YAMAKAWA T, O KURASHIBI, K ISHIKIKA, S KATO, T KODAMA, Y MINDO 1979
Stability of indole-3-acetic acid to autoclaving, aeration and light