Effect of Light of Several Spectral Bands on the Metabolism of Radioactive IAA in Bean Seedlings

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In a study on the effect of light quality on plant growth we found a direct relationship between IAA content and plant height (1). Of the several spectral regions used the red produced plants which were the shortest and had the least IAA. Using low intensity radiation Meijer (3) also obtained inhibition of elongation in red light but was able to obtain some reversal by the addition of IAA and related compounds. Although we did not obtain reversal, a marked difference in the metabolism of exogenously applied IAA in plants exposed to blue and red light was observed. Therefore, this study to compare the metabolism of labeled IAA by plants grown in different regions of the spectrum was undertaken.

Materials and Methods

The characteristics of the light filters and the method of measuring the incident light intensities have been described (1, 7). The range of wavelengths used in this study are referred to by color as given in table 1. The bean seeds were germinated in the dark at 25 ± 1°C and treatments were begun when the seedlings were 5 days old (4–5 cm tall). IAA labeled with C¹⁴ in the 2 position of the side chain was obtained from the California Corporation for Biochemical Research. Ten µl of an aqueous solution containing 0.2 µCi IAA having a specific activity of 13.3 mc/mmol were administered to each bean seedling by injecting 5µl into each cotyledon. After 1 light cycle (8 hr) of different wavelengths, all at a uniform energy of 2900 ergs/cm² per second, radioautograms of a representative plant from each treatment were prepared.

Three intact plants were taken from each of the treatments and the IAA was extracted to yield 3 fractions. Fraction I, designated free IAA, was obtained by methanol extraction (1). Fraction II, assumed to contain bound IAA was obtained by extracting the residue over-night with methanol in a Goldfisch extraction apparatus. The resulting residue was hydrolyzed with 0.5 N HCl for 3 hours to yield a more strongly bound IAA (6) designated fraction III. The volumes of the 3 fractions were reduced to 3 ml in a flash evaporator at 40°C. A 0.1 ml aliquot from each fraction was plated on aluminum planchets and the radioactivity determined in a gas flow counter equipped with a micromil window. Aliquots of 0.2 ml of each fraction were chromatographed on Whatman No. 1 paper using isopropanol : ammonia (7%) : water (8:1:1) as the developing solvent. The chromatograms were scanned with a 4π chromatogram scanner and then used for the preparation of radioautograms. The radioactive metabolites were eluted with methanol, concentrated and rechromatographed in 5 solvent systems (1, 5, 6). The Rf values of the metabolites were compared, with those obtained for synthetic IAA, indoleacetaldehyde and indole acetylaspatic acid.

Results

A typical radioautogram of a bean plant injected with radioactive IAA and exposed to 1 cycle of light is presented in figure 1. It is evident that the label was translocated to regions both above and below the cotyledons. Radioautograms developed from plants which were quick frozen either in the deep freeze (−30°C) or by dipping in isopentane-dry ice (−80°C) showed the same distribution of label. There was no visible difference of translocation in plants exposed to light of different spectral bands.

Dark treated plants yielded 91% of the activity in fraction I whereas red treated plants had only 41% of the total activity in this fraction. In fraction II the yields were reversed with red treated plants having 31% of the activity as compared to the dark which had 11% (table 1).

It is evident from the results that relatively little activity was recovered in fraction III. The total activity recovered from the dark plants was 94%. In the red treated plants in spite of the 3 extractions only 79% of the activity could be recovered. An initial comparison of the blue and red light treated plants showed that in both fractions I and II, besides IAA there were 2 additional radioactive metabolites having similar Rp values. The one having an Rp of 0.02 in isopropanol : ammonia : water

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2 This work was supported by research grants from the University of Alberta and the National Research Council of Canada.
Fig. 1. Radioautogram of a bean seedling showing translocation of radioactivity. IAA-C\textsuperscript{14} was injected into the cotyledons and the seedlings were exposed to 8 hours of light.

Fig. 2. Radioautograms of chromatograms of IAA-C\textsuperscript{14} and of metabolites in fraction I extracted from bean seedlings injected with IAA-C\textsuperscript{14}. The chromatograms were developed in isopropanol: ammonia (7\%): water (8:1:1).

was designated X. When X was chromatographed in other solvents, it yielded 2 spots (X and X\textsubscript{1}). The other unknown having an R\textsubscript{F} of 0.84 was designated Y. The metabolites contributing to the low activity in fraction III could not be detected by the scanner. Figure 2 shows the similarity between IAA in one of the fractions and synthetic IAA and the positions of the metabolites X and Y. More IAA was recovered from fraction I of the blue treatment than of the red. There was very little difference between the amounts of X in the red and blue but there was almost twice the amount of metabolite Y in fraction I of the red than of the blue treatment (table II). The relative activities in fraction II followed a similar pattern.

On the basis of the R\textsubscript{F} values in different solvent systems, table III, one of the metabolites (Y) has been tentatively identified as IAAl since its R\textsubscript{F} values compare closely with those of the synthetic compound.

The R\textsubscript{F} (0.02-0.05) of X was similar to IAAAsp in isopropanol: ammonia: water but in the other solvent systems X separated into 2 distinctly separate spots, X and X\textsubscript{1}, neither of which compared to the R\textsubscript{F} of synthetic indoleacetylaspatic.

**Discussion**

Although the concentrations of labeled compounds in separate portions of the bean plants were not determined, it is clear from the radioautograms that label was translocated to the apical as well as the basal regions. Endogenous IAA or its metabolite may be translocated in a similar fashion from the

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**Table I. Effect of Light of Several Spectral Bands on the Recovery of Radioactivity from Bean Seedlings Injected with IAA-C\textsuperscript{14}**

Each seedling was injected with 0.2 \mu g IAA. These data are the means of 3 experiments.

<table>
<thead>
<tr>
<th>Light transmission (\textmu m)</th>
<th>Fraction I</th>
<th>Fraction II</th>
<th>Fraction III</th>
<th>Total recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark</td>
<td>170,340 (81)</td>
<td>23,780 (11)</td>
<td>4,898 (29)</td>
<td>199,018 (94)</td>
</tr>
<tr>
<td>Blue</td>
<td>159,230 (75)</td>
<td>24,810 (12)</td>
<td>9,013 (45)</td>
<td>193,053 (91)</td>
</tr>
<tr>
<td>Yellow</td>
<td>126,990 (60)</td>
<td>37,990 (17)</td>
<td>10,252 (59)</td>
<td>174,730 (82)</td>
</tr>
<tr>
<td>Red</td>
<td>81,940 (41)</td>
<td>64,520 (31)</td>
<td>14,871 (71)</td>
<td>167,331 (79)</td>
</tr>
<tr>
<td>Red-Far-Red</td>
<td>139,490 (66)</td>
<td>28,410 (13)</td>
<td>9,982 (5)</td>
<td>177,882 (84)</td>
</tr>
</tbody>
</table>

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**Table II. Relative Activity of Metabolites in Extracts of Bean Seedlings Injected with Radioactive IAA-C\textsuperscript{14} then Exposed to 8 Hours of Light**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fraction I</th>
<th>Metabolite (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue</td>
<td>1</td>
<td>IAA 59, X 26, Y 15</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>IAA 52, X 39, Y 9</td>
</tr>
<tr>
<td>Red</td>
<td>1</td>
<td>IAA 41, X 28, Y 31</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>IAA 45, X 36, Y 19</td>
</tr>
</tbody>
</table>
cotyledons which have been shown to contain a high level of IAA.

The metabolism of applied IAA was found to be different for plants grown in different regions of the spectrum. This may account for the different levels of endogenous IAA obtained when plants were grown under different wavelengths. When some of the metabolic data in table I are compared with data from a previous study (1), a direct relationship between label in the most readily extractable fraction, fraction I, and length of hypocotyls or epicotyls and concentration of endogenous IAA (methanol extractable) is apparent (table IV). On the other hand, there is an apparent inverse relationship between fraction II and length of hypocotyls, epicotyls and levels of endogenous IAA.

Although the products of photodegradation of IAA are not completely known, the appearance of indolealdehyde has been demonstrated in the riboflavin-sensitized system (2). In fact Wightman (6) and Pilet (4) in studies on the metabolism of indole compounds have reported indolealdehyde to be one of the labeled intermediates. In the present study, metabolite Y has been tentatively identified as indolealdehyde. In both fractions I and II this compound has approximately twice the concentration in the red treatment as in the blue. It can therefore be speculated that in some way red light stimulates the oxidation of IAA to indolealdehyde much more readily than does the blue. This may be an explanation for the lower levels of endogenous IAA in red light treated plants.

Since our studies show a relationship between light, endogenous levels of IAA and the metabolism of IAA, it is suggested that IAA contributes to the morphogenetic responses observed. However, clear cut evidence for a cause-effect relationship has yet to be provided.

Summary

The metabolism of applied indoleacetic acid-$^{14}$C was different in bean plants grown under various regions of the spectrum with less label being recovered from the red than the blue-grown plants. Besides indoleacetic acid there were 3 additional labeled metabolites one of which had the same $R_f$ values as indolealdehyde in 5 solvent systems. The concentration of this metabolite in the red-grown plants was approximately twice that found in the blue. A relationship between elongation and metabolism of radioactive indoleacetic acid in plants exposed to different wavelengths of light was apparent.

Acknowledgment

The sample of authentic indoleacetic acid was kindly supplied by Dr. W. A. Andraea, C. D. A. Research Institute, London, Ontario.

Literature Cited

Studies on the Inhibitor Resistant Respiration of the Fungus Myrothecium verrucaria

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Among aerobic organisms, and especially among the plants, there are a number of examples of tissues whose respiration is insensitive to the action of the classical inhibitors of cytochrome oxidase: cyanide, azide, and carbon monoxide. Among these can be listed the spadix of Arum (15), Simplocarpus (13), and other aroids (26), potato tuber tissue aged in water (14, 16), some strains of Ustilago (12) and a number of other examples. In no case, however, has there been any satisfactory explanation of the basis for these inhibitor insensitivities. The present paper is concerned with another such system, that of the imperfect fungus Myrothecium verrucaria.

The knowledge of Myrothecium as an inhibitor-insensitive system dates from the work of Darby and Goddard (7) in 1950, who showed that the mycelium of this fungus was insensitive to these inhibitors in concentrations which would inhibit normal systems. They also noted that, like the other insensitive systems, they could extract cytochrome c oxidase activity from the mycelium, and that this extracted activity was insensitive to these inhibitors, although the respiration of the intact mycelium was not. The present paper is a continuation of that study.

Materials and Methods

The cultures of Myrothecium verrucaria used in this work were obtained through the courtesy of R. T. Darby of the Quartermaster General Laboratories, Natick, Massachusetts. The mycelium was grown in liquid culture on a rotary shaker at 25° in the following medium (8): 0.012 M K2HPO4, 0.020 M KH2PO4, 0.038 M NH4NO3, 0.009 M MgSO4, 2.0% (w/v) glucose, and 5% malt extract (Difco). Using Erlenmeyer flasks and a shake rate of about 200 rpm, a 2.5:1 ratio of flask capacity to fluid volume was found to insure adequate surface for aeration. When heavily inoculated with a spore suspension, these conditions gave satisfactory mycelial growth without sporulation in 48 hours, and this culture age was used for all experiments except where noted. The colonies, consisting of mycelial pellets about 0.4 mm in diameter, were harvested by filtration from the medium, and were washed with 0.032 M potassium phosphate buffer, pH 7, before use.

To obtain spores, the same medium without glucose or malt extract was solidified with 2% agar in petri dishes, and covered with a piece of Whatman No. 1 filter paper. Upon inoculation by flooding with a spore suspension, a sparse mycelial growth followed by copious sporulation was observed in 5 to 12 days at room temperature. The spores could then be washed off the plate with sterile water, and stored in the cold for at least 1 month with no loss in viability (9).

Mitochondria were prepared from the fungal colonies by mechanical disruption and differential centrifugation at 0°. The colonies were suspended in a medium consisting of 0.5 M mannitol, 0.012 M

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1 Received November 12, 1964.
2 This investigation was carried out during the tenure of a United States Public Health Service predoctoral fellowship to George W. Kidder III, and is a part of a doctoral dissertation presented by him to the Graduate School of Arts and Sciences of the University of Pennsylvania, February, 1961.
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