INFLUENCES OF GIBBERELLIC ACID ON METABOLISM OF INDOLEACETIC ACID, ACETATE, AND GLUCOSE IN ROOTS OF HIGHER PLANTS

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The major effects of the gibberellins (GA) on plants have been reviewed recently (13, 14) but no definite linkage with any metabolic pathway has been established. In their study of photosynthesis in giberellin treated leaves Haber and Tolbert (8) concluded that giberellin acid neither enhanced the rate of CO₂ fixation per unit of leaf tissue nor altered the general pathways of short time metabolism of the newly fixed C₁₄O₂ in the sugars, organic acids, and amino acid products. In long term experiments, great changes in carbohydrate contents of giberellin treated plants have been reported (4, 9). Inhibition in the level of indoleacetic acid oxidase by giberellin acid has been reported by Pilet (12). However, Brian and Hemming (5) did not find a similar effect in their system. Recently, Galston and Warburg (7) suggested a possible action of giberellin through an auxin-sparing mechanism by way of the formation of IAA oxidase inhibitor. It is the purpose of this paper to describe the results of experiments on the influence of GA on plant metabolism. In the method used, plant tissue was first pre-treated with GA and then incubated with a selected isotopically labeled substrate. Samples of the tissue were then harvested after several hours of incubation. The influence of GA on the metabolism of the material was determined by comparing the extents of the labeled atoms which have been metabolized and incorporated into other compounds between the control and treated tissues.

MATERIALS AND METHODS

Alaska pea (Pisum sativum L.) and sweet corn (Zea mays L. var. Golden Cross) were soaked in distilled water for 2 hours and were then germinated in the dark between two sheets of moist paper. Uniform seedlings were removed from the paper after two or three days and the roots were immersed in either 0.01 M phosphate buffer (pH 5.2) or buffer + 5 ppm GA. At the end of the 2 hours pre-treatment period, the seedlings were removed from the solution and then rinsed with water. The distal 2 cm of the roots was cut off, blotted dry, and weighed. Equal samples of root tips were placed in 50 ml Erlenmeyer flasks which contained 10 ml of 0.01 M phosphate buffer, pH 5.2, along with C¹⁴ labeled substrates, and were allowed to incubate at 26 to 28° C. The respiratory C¹⁴O₂ was collected periodically as described previously (6). At the end of the incubation period, the tissues were removed from the medium, rinsed with water, and homogenized with ethyl alcohol. The radioactivities of the medium, alcohol extract of the tissues, and the residue of the tissue were also determined by the usual method.

The incorporation of C¹⁴ in each isotopically labeled metabolite was studied by paper chromatographic and radioautographic techniques.

RESULTS AND DISCUSSION

Effect of GA on Metabolism of IAA-1-C¹⁴
The influence of GA on the metabolism of auxins in higher plants has always been of interest. This problem was approached by using C¹⁴ labeled indoleacetic acid. Corn root tissue has been chosen for this study because in addition to the decarboxylative oxidation, C-1 carbon of IAA has been found to incorporate into many metabolites. The root tips from control and GA treated seedlings were incubated separately at 26° C in 10 ml 0.01 M phosphate buffer (pH 5.2) containing 2.0×10⁻³ M IAA-1-C¹⁴. Twelve 40-watt fluorescent lights placed 3 ft from the flask, providing a light intensity of 800 ft-c, were used for illumination during the incubation period. Respiratory CO₂ was collected periodically and precipitated as BaCO₃. After a 6 hr period of incubation, the root tissues were removed, rinsed, and homogenized with 10 ml ethyl alcohol. The alcohol extract was filtered and the residue was washed thoroughly with alcohol. The radioactivities of BaCO₃, alcohol extract, and insoluble residue were determined.

The data from two separate experiments are shown in table I. There was no significant difference in the total absorption of IAA by the control and GA treated tissues. However, the ratio (O/A) between the decarboxylative oxidation and the absorption of IAA was slightly higher in GA treated tissues indicating that oxidative degradation of IAA might be affected. The time course radiochemical recovery of respiratory CO₂ revealed that a noticeable increase in oxidation by GA treated tissue occurred only during the first 2 hours. The cumulative recoveries of respiratory CO₂
from control and GA treated tissues were quite similar
(fig 1). The incorporation of isotopic carbon from
IAA-1-C14 into various metabolites as revealed from
paper chromatographic and radioautographic studies
was presented in table II. Results indicated that the
formation of indoleacetylaspartate was significantly
reduced. The free IAA-1-C14 concentration as cal-
culated from the experimental data was 15 % higher in
GA treated tissue (3.07 μg/1 gm fresh tissue) than in
the control tissue (2.69 μg/1 gm fresh tissue). If
the concentration of absorbed IAA in tissue could be
an indication for the endogenous auxin level, then
higher endogenous IAA in GA treated tissue would
be expected. This observation provides further evi-
dence in support of auxin-gibberellin interaction. It
was not in complete agreement with the report by
Pilet (12), however, that the increase of IAA level
in GA treated tissue was a result of inhibition of IAA
oxidase but rather from a result of the decreasing
formation of indoleacetylaspartate. Hayashi and
Murakami (9) in bioassays of extractable and dif-
solvable auxins from several different plants, could
find no change in auxin levels after GA treatments.
Since indoleacetylaspartate is as effective as IAA either
in curvature or in elongation tests with oat coleoptiles
(1), measurements of extractable and diffusable
auxins by a bioassay method would not differentiate
this transformation and the concentration of each.
No difference was observed in other radioactive meta-
bolites between the control and GA treated tissues.

Effects of GA on glucose metabolism Several
experiments similar to those described in the pre-
ceding section were also carried out with pea root
sections with either glucose-U-C14, glucose-1-C14 or
glucose-6-C14 as substrate at a concentration of 1 mg
glucose per 10 ml medium. The root tissues (1.3-1.4
gm fresh weight) were incubated for 6 hours. At
the end of incubation, the reaction was stopped by adding
0.5 ml 6 N H2SO4. Shaking was continued for an-
other 20 minutes in order to remove completely the
respiratory CO2. Tissues were harvested, rinsed, and
ground with ethyl alcohol. Radioactivities of respira-
tory CO2, alcohol extract, and alcohol insoluble resi-
due were determined. The alcohol extracts were
chromatographed unidimensionally with n-butanol-

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**TABLE I**

**Effect of GA on Distribution of Radioactive Carbon from IAA-1-C14 by Corn Root Tips**

<table>
<thead>
<tr>
<th>Expt</th>
<th>Tissue wt</th>
<th>Treatment</th>
<th>Total Absorption IAA-1-C14</th>
<th>O/A</th>
<th>% Recovery of C14 in various fractions from absorbed IAA-1-C14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg</td>
<td>μg</td>
<td></td>
<td></td>
<td>% Recovery of C14 in various fractions from absorbed IAA-1-C14</td>
</tr>
<tr>
<td>1</td>
<td>651</td>
<td>Buffer</td>
<td>18.9</td>
<td>0.50</td>
<td>50.2</td>
</tr>
<tr>
<td>2</td>
<td>657</td>
<td>GA + Buffer</td>
<td>18.6</td>
<td>0.53</td>
<td>52.8</td>
</tr>
</tbody>
</table>

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**Table II**

**Paper Chromatographic Study of Formation of Radioactive Metabolites in Corn Root Tissues Incubated in IAA-1-C14 Solution**

<table>
<thead>
<tr>
<th>Distribution of radioactivity</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B*</td>
</tr>
<tr>
<td>R1O</td>
<td>0.03</td>
</tr>
</tbody>
</table>

**Table III**

**Respiratory Stress in Plants**

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**Fig. 1.** Time course plot of radiochemical recovery in respiratory CO2 from indoleacetic acid-1-C14 by control and GA treated corn root tissues.
acetic acid-water solvent (4:1:1 v/v). Radioautograms of the resultant chromatograms revealed the presence of three principal components. These components appeared identical with respect to Rf values and relative intensities as between the GA treated and non-GA treated tissues. Table III shows the results on total absorption of substrate, and C\(^{14}\) recoveries in respiratory C\(^{14}\)O\(_2\), alcohol insoluble tissue constituents, and alcohol extract. Five independent experiments were carried out with glucose-U-C\(^{14}\) and three experiments each with glucose-1-C\(^{14}\) and glucose-6-C\(^{14}\). The results of these experiments were in close quantitative agreement throughout and therefore the average values are presented for comparison. It is apparent from the data of Table III that there is no significant difference between the control and GA treated tissues either on the total absorption of substrate or in the percent distribution of C\(^{14}\) in various fractions. It also is apparent that an appreciable portion of the labeled carbon supplied to pea root tissue as glucose becomes incorporated into cell wall constituents (residue insoluble in 80 % ethyl alcohol). Percentagewise, the anabolic functions of C-6 carbon from glucose as measured directly from its incorporation to cellular constituents was higher (47 %) than that of C-1 carbon (34 %). In contrast, the participation of the catabolic functions of carbon 1 of glucose as measured from the radioactivity of respiratory CO\(_2\) revealed that it was 20 % greater than the carbon 6. Approximately 10 % of the radioactivity from the absorbed glucose in root tissue can be extracted by 80 % ethanol from which three prominent components including the unchanged glucose have been revealed by paper chromatography and radioautography. The average value of C\(_6\)/C\(_1\) ratio derived from respiratory CO\(_2\) from control pea root tissue is 0.68 ± 0.03 (3 runs) which is in agreement with the value (0.74 ± 0.11) as reported by Humphreys and Dugger (10). Pre-treatment of pea roots with GA did not alter the C\(_6\)/C\(_1\) (0.70 ± 0.05) appreciably, thus indicating that the pathways of glucose catabolism are not affected. When the yields of C\(^{14}\) incorporation into cell wall constituents from C-1 and C-6 of glucose were compared, the result again indicated that no appreciable differences were found between the control tissue (1.47) and GA treated ones (1.31). It is therefore concluded that a short term GA treatment of pea roots will neither alter their pathways of glucose catabolism nor exert any gross effect in the incorporation of carbon atoms of glucose into other major cellular constituents. The time course recovery of radioactivity in respiratory CO\(_2\) from the control and the GA treated pea root tissues metabolizing C\(^{14}\) labeled glucose were shown in figure 2 which provided further evidence that no significant effect either on the combustion of C-1 or C-6 of glucose was observed at any given time during the ex-

**Table III**

**Effect of GA on Uptake and Utilization of Glucose by Pea Root Tips**

<table>
<thead>
<tr>
<th>C(^{14}) Substrate</th>
<th>Treatment</th>
<th>No. Runs</th>
<th>Weight Tissue Used</th>
<th>Total Absorption of Glucose</th>
<th>Recovery of C(^{14}) in Various Fractions from Absorbed Glucose</th>
<th>Respiration CO(_2)</th>
<th>Alcohol Extract of Tissue</th>
<th>Alcohol Insoluble Residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose-U-C(^{14})</td>
<td>Buffer</td>
<td>5</td>
<td>1.402 ± 0.043</td>
<td>54.1 ± 4.4</td>
<td>43.4 ± 5.8</td>
<td>10.8 ± 2.7</td>
<td>45.8 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>Glucose-U-C(^{14})</td>
<td>GA + buffer</td>
<td>5</td>
<td>1.387 ± 0.037</td>
<td>51.3 ± 2.8</td>
<td>41.8 ± 2.9</td>
<td>13.1 ± 3.8</td>
<td>45.1 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>Glucose-1-C(^{14})</td>
<td>Buffer</td>
<td>3</td>
<td>1.394 ± 0.002</td>
<td>66.0 ± 3.6</td>
<td>57.8 ± 2.2</td>
<td>9.4 ± 1.7</td>
<td>32.8 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>Glucose-1-C(^{14})</td>
<td>GA + buffer</td>
<td>3</td>
<td>1.396 ± 0.003</td>
<td>63.5 ± 3.3</td>
<td>56.2 ± 2.8</td>
<td>8.5 ± 0.8</td>
<td>35.3 ± 2.4</td>
<td></td>
</tr>
<tr>
<td>Glucose-6-C(^{14})</td>
<td>Buffer</td>
<td>3</td>
<td>1.396 ± 0.002</td>
<td>55.2 ± 2.8</td>
<td>39.1 ± 2.4</td>
<td>12.6 ± 2.3</td>
<td>48.3 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>Glucose-6-C(^{14})</td>
<td>GA + buffer</td>
<td>3</td>
<td>1.395 ± 0.002</td>
<td>53.5 ± 2.9</td>
<td>39.2 ± 1.3</td>
<td>14.5 ± 1.5</td>
<td>46.3 ± 1.5</td>
<td></td>
</tr>
</tbody>
</table>
Experimental period. Furthermore, it was noted that the Ca/C1 ratios during the first 2 hours of incubation were near unity, indicating that glucose was catabolized in these tissues mainly via the glycolytic path (1st hr 1.07, 2nd hr 0.95). However, the Ca/C1 ratios which calculated from the yields of CO2 between the third and the sixth hours showed a decrease from 0.68 at 4th hour to 0.63 at 6th hour, suggesting that a shift of the catabolic pathways had taken place, presumably an increase of participation of pentose cycle route.

Effects of GA on Acetate Metabolism Acetate is metabolized by plant tissues via several pathways (3), the carboxyl carbon of acetate appeared rapidly in the Krebs cycle acids (11), and Krebs cycle related amino acids (2). It is also incorporated into the lipides of the Avena coleoptile (3). To investigate the influence of GA on acetate metabolism, the pea root tissues (control and GA treated) were incubated separately in phosphate buffer, pH 5.2, containing 0.5 mg sodium acetate (acetate-1-C14 or acetate-2-C14) for 4 hours at 26° C. The respiratory 14O2 was trapped in 0.5 M NaOH solution and determined hourly. At the end of a 4 hour incubation period, the tissues were harvested, rinsed, and ground with 80% alkali ethanol to assure that no acetate in the tissue would be lost. The radioactivity of BaCO3, alcohol extract and alcohol insoluble residue was determined in the usual manner. After the initial counting, the plant containing alcohol extract was acidified with a small amount of acetic acid and allowed to dry completely in order to remove the radioactive acetate. The plantchett was then again counted. The difference in radioactivity was recorded as free acetate. The alcohol extract was subjected to ascending paper chromatographic separation. The n-propanol-ethanol-ammonium carbonate buffer solvent system was employed (15). The radioautographs prepared from the paper chromatograms revealed the detail incorporations of isotopic carbon from either acetate-1-C14 or acetate-2-C14, but in no case were there any gross differences found between GA treated and control tissues. Therefore, it is evident that GA does not exert any important effect on the conversion of acetate carbon into any of the major products of acetate metabolism. Six separate experiments were carried out with acetate-1-C14 and four experiments with acetate-2-C14. The average values of total absorption, respiratory CO2, alcohol insoluble residue, and alcohol extract are shown in Table IV. The total uptake of acetate by GA treated tissue was consistently lower than that of the control, although the differences were small. The oxidation of either C1 or C2 carbons of acetate was slightly increased in GA treated tissue. The catabolic functions of C-1 carbon of acetate were much greater than that of C-2 as indicated from the percent radiochemical recovery in respiratory CO2. Again, it was noted that considerably more activity has been incorporated into cellular constituents from C-2 carbon of acetate, indicating that the participation of C-2 of acetate in direct biosynthetic functions was greater than that of C-1. GA treatment of pea root tissues revealed a slight increase in the utilization of acetate. Since results from several runs did not show any change, this small increase may not be considered significant.

Summary

The effects of gibberelic acid on the metabolism of indoleacetic acid, glucose and acetate in roots of higher plants have been studied by using C14 labeled compounds.

GA, at the concentration of 5 ppm increased slightly C14O2 production from administered IAA-1-C14 in corn root tissues. The formation of indoleacetyspartate was greatly inhibited and consequently the free IAA level in GA treated tissue was increased by 15% over the control tissues.

The catabolic pathways of glucose and acetate in pea root tissue as examined by the tracer method were not found to be affected to any significant extent by short-term GA treatment. However, GA treatment slightly increased the catabolic oxidation of C-1 and C-2 carbons of acetate.

Table IV

Effect of GA on Uptake of Utilization of Acetate by Pea Root Tips

<table>
<thead>
<tr>
<th>C14 Substrate</th>
<th>Treatment</th>
<th>No. runs</th>
<th>Weight of tissue used</th>
<th>Total respiratory CO2</th>
<th>Alcohol extract of tissue in %</th>
<th>Free acetate in %</th>
<th>Alcohol insoluble residue in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Acetate-1-C14</td>
<td>Buffer</td>
<td>4</td>
<td>992 ± 6</td>
<td>50.4 ± 4.8</td>
<td>55.0 ± 0.9</td>
<td>11.4 ± 0.6</td>
<td>14.2 ± 1.0</td>
</tr>
<tr>
<td>*Acetate-1-C14</td>
<td>GA + buffer</td>
<td>4</td>
<td>974 ± 22</td>
<td>45.6 ± 2.0</td>
<td>62.6 ± 3.3</td>
<td>24.2 ± 2.2</td>
<td>13.2 ± 0.7</td>
</tr>
<tr>
<td>Acetate-1-C14</td>
<td>Buffer</td>
<td>2</td>
<td>1,117</td>
<td>75.7</td>
<td>47.5 ± 0.5</td>
<td>32.3 ± 1.5</td>
<td>28.2 ± 1.9</td>
</tr>
<tr>
<td>Acetate-1-C14</td>
<td>GA + buffer</td>
<td>2</td>
<td>1,066</td>
<td>72.4</td>
<td>49.6 ± 0.4</td>
<td>27.8 ± 0.5</td>
<td>22.7 ± 1.0</td>
</tr>
<tr>
<td>Acetate-2-C14</td>
<td>Buffer</td>
<td>4</td>
<td>1,105 ± 8</td>
<td>70.7 ± 4.3</td>
<td>27.2 ± 7.2</td>
<td>42.9 ± 8.2</td>
<td>29.8 ± 2.0</td>
</tr>
<tr>
<td>Acetate-2-C14</td>
<td>GA + buffer</td>
<td>4</td>
<td>1,103 ± 13</td>
<td>63.9 ± 5.1</td>
<td>29.8 ± 5.1</td>
<td>35.8 ± 6.4</td>
<td>34.0 ± 2.6</td>
</tr>
</tbody>
</table>

* Reaction was stopped by adding H2SO4 after 4 hrs.
Chromatography and radioautography of the alcohol soluble constituents of pea root tissues which had been incubated with either acetate-1-C\textsuperscript{14}, acetate-2-C\textsuperscript{14}, or glucose-U-C\textsuperscript{14} failed to reveal any major effect of short-term GA treatment although the isotopic carbon of all three substances was rapidly incorporated into a variety of compounds in the plant.

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**Literature Cited**


