Glucose Metabolism of Various Tissues of Pear Buds

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ABSTRACT
Various tissues in flower buds of Pyrus calleryana Decne., differ in their metabolic activity. Brown outer scales utilized more exogenously supplied glucose, particularly through the pentose phosphate pathway, than did the central axes and the green inner scales. They also contained more endogenous reducing sugars, and glucose leaked out more readily from the brown scales than from the other tissues. In contrast, respiration of the central axes was nine times as great as that of the brown scales, and two to four times as much glucose was metabolized through glycolysis. Membranes of the central axes were less permeable to glucose. Because the brown scales are 75% of the dry weight of the bud, they dominate its pattern of glucose metabolism.

The resting flower bud of a tree is a complex organ composed of at least three different tissues: the brown outer scales; the green inner scales; and the central axis. In studying metabolism of buds, most investigators have used the entire structure and found that the metabolism resembled that of old rather than of young tissues. Thorn's work (17), which included a comparison of bud parts, is a notable exception.

In developing a procedure for extracting RNA from pear buds, we found that unknown substances in the brown scales interfered with the extraction of nucleic acids from the central axes. This indication of a striking difference between the different bud components, together with the unexpected metabolic pathways of glucose utilization (19) in the presumably young tissues, led us to re-examine the metabolism of pear buds. We determined the pathways of glucose metabolism in various parts of the buds and correlated it with another indication of tissue age, namely permeability of cell membranes.

Information describing metabolic changes in pear buds was reviewed earlier (19). Resting whole buds respire mostly through the pentose phosphate pathway (19). High pentose-P pathway activity is characteristic of old plant tissues (3, 5, 9, 12, 16, 18). Buds also contain a substantial amount of fatty material (6). In the bud cells the deposition of a lipoid layer on the surface of the protoplasm is associated with the separation of the protoplasm from the cell walls (7). In the plants kept the rest period the protoplasm absorbs water and swells (6, 19). Glucose metabolism changes during swelling. The contribution of the pentose-P pathway to respiration decreases and the role of the Embden-Meyerhof-Parnas glycolytic pathway increases during this time (19). The change in water content of buds during swelling indicates that resting buds are relatively dry. Dehydrated tissues have been shown to respire more through the pentose-P pathway (11), and this can be accepted as an explanation for the relatively high pentose-P pathway activity in resting buds. The state of dehydration may mimic the effect of the age of the tissues with respect to their respiratory pattern.

Another measure of cell age is the permeability of cell membranes. Increases in permeability of cell membranes with age were observed in apples (4), avocados (15), bananas (2), and bean endocarp (10) and are considered normal for senescing tissues (13).

MATERIALS AND METHODS

Plant Material. Terminal flower buds from two 7-year-old trees of Pyrus calleryana Decne. were collected between mid-January and mid-March 1970. The upper portion of both trees had flowered at least four times before the start of the experiment. We cut shoot tips from the tree 5 to 8 feet above ground and brought them into the laboratory. There we excised the terminal buds and stripped away all the bud scales. The outermost two or three scales were discarded and the remaining scales were separated into groups of brown (upper portion, mostly outer) scales and pale green (lower portion, mostly inner) scales. The central axes of the buds were used separately.

Total respiration of whole buds, central axes, green scales, and brown scales was determined in a Gilson4 differential respirometer. Oxygen uptake was measured at 30-min intervals during 2-hr incubation in 0.2 M potassium phosphate buffer, pH 5.7, at 25 C and calculated as μl/g fresh weight-hr.

Glucose metabolism of the central axes, brown scales, and green scales (minimum weight of 50, 50, and 30 mg, respectively) was measured in an 8-unit radiometric respirometer (1). To each flask, with sample included, we added 10 ml of phosphate buffer and 1 μC of labeled glucose of either glucose-U-14C or glucose-6-14C. Specific radioactivities of the sugars were 5.09 mc/mmole for G-1-14C and 4.58 mc/mmole for G-6-14C. The C1:C2 ratio, total uptake, respired 14CO2, and amount of label incorporated into ethanol-insoluble residue were determined as described earlier (19).

In a separate series of experiments, tissues were incubated in 9 ml of 10-3 M glucose solution for 3 hr before determining the glucose metabolism. After preincubation, 1 ml of 10-3 M glucose containing 1 μC of glucose was added and the uptake, respiration, and incorporation of glucose were determined as before (19).

Membrane permeability was determined by the uptake of 14C-glucose with or without preincubation in glucose solution and by the leakage of 14C-glucose from the cells.

For uptake studies we incubated 10 samples of each tissue in perforated crucibles in a shallow dish containing 200 ml of 10-3 M glucose which included 20 μC of G-1-14C. After 5, 10, 15, 30, and 60 min of incubation, two samples each of central axes and

1 Mention of a trademark name or a proprietary product does not imply its approval by the United States Department of Agriculture to the exclusion of other products that may also be available.

2 Abbreviations: G-1-14C: glucose-1-14C; G-6-14C: glucose-6-14C.
The incubation had leaked was pipetted central incubating flasks containing incubation ment samples were homogenate extracted and axes, green metrically by the brown scales being much reducing and four central axes and brown scales for 3 hr. The leakage of glucose was transferred.

As a modification of this experiment we preincubated the bud parts in unlabeled 10−4 M glucose for 3 hr. The rest of the experiment was as described above.

We measured the leakage of glucose from the bud parts by incubating central axes, green scales, and brown scales for 3 hr in flasks containing 10 ml of 10−4 M glucose with 1 μC of G-1-14C. After incubation the tissues were rinsed and transferred to flasks containing 10 ml of distilled water. A 1-ml aliquot from each flask was pipetted into a planchet 5, 10, 15, 30, and 60 min after the samples were transferred. After the water was evaporated, the aliquots were counted to determine the amount of label which had leaked from the tissue.

Reducing sugars in the bud tissues were determined colorimetrically by the method of Nelson (14). Samples of central axes, green scales, and brown scales were ground in a mortar and extracted in 80% (v/v) ethanol in a VirTis 45 homogenizer. The homogenate was filtered, and the extract was made up to 10 ml with 80% ethanol. The amount of reducing sugars was calculated using glucose as a standard.

RESULTS

The three components of the buds differed greatly in weight per bud, respiration, content of reducing sugars, pathways of glucose metabolism, and utilization and incorporation of labeled glucose. More than half the fresh weight of the buds was brown scales and about one-fourth was central axes (Table I). The central axes had the highest rate of respiration (O2 uptake) and the brown scales and whole buds the lowest, with the green scales being intermediate. The brown scales contained twice as much reducing sugar as the central axes per gram fresh weight and four times as much per bud.

The brown scales utilized the most labeled glucose, incorporated the largest percentage into ethanol-insoluble residue, and respired the largest amount of 14CO2 (Table II). The C6:C1 ratio was equally low for the brown and green scales but it was much higher for the central axes (Table II). Preincubation of tissues in glucose decreased the uptake of labeled glucose by the brown scales but did not change the uptake by the other two tissues. The decrease of uptake in the preincubated brown scales substantially affected the incorporation of label into ethanol-insoluble residue and the total amount of label utilized, but it did not affect the percent released as CO2 (Table II).

Table I. Weight, Respiration, and Reducing Sugar Content of Different Tissues of Flower Buds of Pyrus calleryana

<table>
<thead>
<tr>
<th>Part of Bud</th>
<th>Fresh Weight</th>
<th>Dry Weight</th>
<th>Respiration (μl O2/g fresh wt)</th>
<th>Reducing Sugars (mg/g fresh wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central axis</td>
<td>16.1</td>
<td>4.8</td>
<td>1600</td>
<td>6.9</td>
</tr>
<tr>
<td>Green scales</td>
<td>10.0</td>
<td>3.6</td>
<td>860</td>
<td>4.9</td>
</tr>
<tr>
<td>Brown scales</td>
<td>31.9</td>
<td>25.9</td>
<td>180</td>
<td>15.0</td>
</tr>
<tr>
<td>Total per bud</td>
<td>58.0</td>
<td>34.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Mean of 15 buds collected March 3, 1970.

Table II. Metabolism of Glucose-1-14C and Glucose-6-14C by Different Tissues of Flower Buds of Pyrus calleryana

<table>
<thead>
<tr>
<th>Part of Bud</th>
<th>C6:C1 Ratio</th>
<th>14CO2 Respired</th>
<th>14C in Ethanol-Insoluble Residue</th>
<th>Label Utilized</th>
<th>Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C1</td>
<td>C4</td>
<td>C1</td>
<td>C4</td>
</tr>
<tr>
<td>Without incubation</td>
<td></td>
<td>0.41</td>
<td>0.39</td>
<td>26.3</td>
<td>10.6</td>
</tr>
<tr>
<td>Central axis</td>
<td>0.16</td>
<td>0.19</td>
<td>23.3</td>
<td>4.4</td>
<td>3.2</td>
</tr>
<tr>
<td>Brown scales</td>
<td>0.10</td>
<td>0.16</td>
<td>43.3</td>
<td>6.8</td>
<td>13.9</td>
</tr>
<tr>
<td>With incubation</td>
<td></td>
<td>30.7</td>
<td>3.4</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Central axis</td>
<td>24.7</td>
<td>2.1</td>
<td>1.6</td>
<td>190</td>
<td></td>
</tr>
<tr>
<td>Green scales</td>
<td>41.3</td>
<td>6.7</td>
<td>1.0</td>
<td>91</td>
<td></td>
</tr>
</tbody>
</table>
GLUCOSE METABOLISM OF PEAR BUDS

Time-course studies of uptake and incorporation of G-1-14C revealed that glucose uptake took place very rapidly in both types of tissues. The brown scales took up more glucose and maintained a higher rate of uptake than did the central axes (Fig. 1). Incubation of tissues in 10-4 M glucose for 3 hr before adding the labeled glucose decreased the uptake initially for both tissues. This effect disappeared after 30 minutes in the central axes (Fig. 1). Incubation also decreased incorporation into ethanol-insoluble residue slightly for both tissues, but the effect was greater on the central axes.

Leakage of labeled compounds also took place rapidly from all tissues. The leakage was greatest from the brown scales, less from the green scales, and least from the central axes (Fig. 2).

DISCUSSION

The large differences in glucose metabolism among components of the pear bud indicate the difficulty of generalizing about metabolic patterns from a study of the whole bud. The different components respired at different rates. The central axes respired at the highest rate and the brown scales at the lowest rate. Utilization of glucose, however, was different. The bud scales took up and respired the most glucose; the central axes respired the least glucose. The brown scales also had the lowest C6:C1 ratio. Although we are aware of difficulties experienced by using the C6:C1 ratios of the respired CO2 as a measure of the pentose-P pathway activity (8), we used this method because of its simplicity. Our measurements indicate that the brown scales were high in pentose-P pathway activity, whereas the central axes utilized glucose more through the Embden-Meyerhof-Parnas pathway.

The uptake and leakage of glucose was much less for the central axes than for the brown scales. Time-course studies indicated that most of the leakage took place within a short period of time. The rapid leakage occurred from the so-called free space (4), indicating that the brown scales possess much more free space and are more permeable to sugars than the central axes. The uptake studies showed a very similar pattern with lesser magnitudes.

The preincubation experiments in 10-4 M glucose revealed that one cannot saturate the relatively small free space of the central axes but that this is possible with the brown scales. The glucose uptake of central axes did not change as a result of preincubation, whereas uptake by the brown scales greatly decreased.

All measures used in this study indicated that there are two patterns of glucose metabolism in the tissues of the pear buds and that these patterns are related to the age of the tissue. Total respiration of young tissue is high, whereas the old tissue respires at a low rate (8). The pentose phosphate activity of young tissue is low, but it increases with age (3, 5, 9, 12, 16, 18). The permeability of young tissue is well controlled, but, with age, the free space of the tissue and its leakiness increase (4, 13). Therefore, we consider the central axes of the pear buds as young tissue and the brown scales as old tissue. Since most of the bud is brown scales (75% of the dry weight), results of metabolic studies using whole buds will be characteristic of those obtained with brown scales. Only central axes should be used to provide the best correlation of metabolic activity of the bud with ontogenetic changes.

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