PRODUCTS FORMED FROM GLYCOLIC ACID IN PLANTS*

BY N. E. TOLBERT† AND MARJORIE S. COHAN‡

(From the Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, Beltsville, Maryland)

(Received for publication, March 6, 1953)

In green plants there is an active enzyme which oxidizes glycolic acid to glyoxylic acid (1); however, in vivo or in crude plant saps the glyoxylic acid does not accumulate in appreciable amounts but is further utilized. In this paper evidence is presented to show that the major end-products from glycolic acid utilization by barley and wheat plants are glycine, serine, and an unknown. The formation of serine appears to be analogous to the recently established pathway in animals and microorganisms for synthesis of serine from glycine (2).

Methods

Glycolic acids labeled with C¹⁴ in either the carboxy or α-carbon atom were used throughout (3). In experiments in vivo, leaves were vacuum-infiltrated with the free acid (4), allowed to stand from a few minutes to 3 hours in the light, and then killed by grinding the tissue in boiling 80 per cent ethanol. Etiolated Pawnee wheat leaves were kept in the dark until the grinding process was carried out. In experiments in vitro, the green barley and wheat leaves were ground in a chilled mortar and then squeezed through cheese-cloth, the sap was adjusted to pH 8.3, and cell fragments were removed by centrifugation. 1 ml. of the cell sap and 2 ml. of a solution containing about 2 mg. of glycolate were incubated in the Warburg flask at 30° with shaking for 1 hour and then poured into boiling 80 per cent ethanol to stop the reaction. Phosphate buffer was omitted, and the calcium of the original glycolate salt was removed with Dowex 50 before use in order to facilitate subsequent chromatography.

Barley chloroplasts and chloroplast fragments were washed twice with distilled water and separated from cytoplasm and mitochondria by differential centrifugation (5). The omission of salts or sugar from the usual procedure for separation of chloroplasts was also necessary because of subsequent chromatography. By working rapidly and in the cold a fair yield

* This work was supported in part by a research grant from the Atomic Energy Commission.
† Present address, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.
‡ Present address, Citrus Experiment Station, University of California, Riverside, California.
of chloroplasts and chloroplast fragments could be obtained. In Warburg manometer respiration experiments they actively catalyzed the oxidation of glycolic acid. Roughly 10 per cent of the total enzyme activity of the plant was separated with the plastids and the remainder was in the cytoplasm. Between pH 4 and 8 the percentage of activity isolated with the chloroplasts and chloroplast fragments was found to be independent of the pH during the separation and of the number of times the particles were washed. This pH independence would indicate that the enzyme activity of the chloroplast fragments may not be due to protein adsorbed on them during the isolation process (5).

The alcoholic solutions were chromatographed, and the products were identified as described by Benson et al. (6), using water-saturated phenol in the first direction and butanol-propionic acid-water in the other direction. Glycine and serine were eluted separately and rechromatographed separately to assure that they were not cross-contaminated. They were identified by the ninhydrin spray test, by their $R_F$ values in two different solvents, and by cochromatography with known amino acids. For degradation purposes, the glycine or serine spots from several chromatograms from one experiment were eluted with water and combined with inactive carrier. The glycine was degraded with ninhydrin (7); each carbon was converted to BaCO$_3$ and its $^{14}C$ activity counted. The degradation of serine was based on the oxidative decarboxylation by periodic acid (8, 9). Both procedures were checked by using known $^{14}C$-labeled compounds, glycine-2-$^{14}C$ and serine-3-$^{14}C$.

**Results**

Products of glycolic acid oxidation *in vivo* and *in vitro* are listed in Tables I and II. In these experiments of an hour’s duration there was almost complete utilization of the glycolic acid. Glycine was an end-product in every case, and the relative amounts formed compared to other products are indicated in Tables I and II. When $\alpha$-labeled glycolic acid was used as the substrate for experiments *in vivo* and *in vitro*, the glycine which was formed was also labeled in the $\alpha$-carbon. The direct conversion of the 2-carbon chain of glycolic acid to glycine was substantiated by other experiments with the carboxy-labeled acid which yielded glycine labeled with $^{14}C$ only in the carboxy carbon. In each case very little $^{14}O_2$ was formed.

Glycolic acid is known to be oxidized first to glyoxylic acid (1), but the present data indicate that, *in vivo* or in plant sap, the glyoxylic acid is rapidly converted to glycine. In experiments *in vitro*, stopped after having been run for only a short period of time, small amounts of glyoxylic acid were present. Its identity was established by $R_F$ values in two different solvents and by cochromatography with inactive glyoxylic acid, in which
there was coincidence of the radioactivity and location of the glyoxylic acid. The latter was detected by acid spray tests on the chromatogram with brom cresol green. In the chromatographic procedure glyoxylic acid had the same $R_f$ value in the butanol-propionic acid-water solvent as glycolic acid, but it hardly moved at all in the phenol-water solvent (6). In experiments in vivo glyoxylic acid as an intermediate was not detected on the chromatograms, but glycine was always present. Even in vitro, the amount of glycine present after short periods far exceeded the amount of glyoxylic acid present.

**Table I**

### Glycine from Oxidation by Plants of Glycolic-2-C¹⁴ Acid

<table>
<thead>
<tr>
<th>Plant material</th>
<th>Relative amount*</th>
<th>Per cent activity</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Carboxyl carbon</td>
<td>$\alpha$-Carbon</td>
</tr>
<tr>
<td>In vivo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green wheat leaves</td>
<td>++</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Etiolated wheat leaves</td>
<td>++</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Green barley leaves</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In vitro</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green wheat sap</td>
<td>++</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Etiolated wheat sap (15 min.)</td>
<td>+++</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>&quot; &quot; (180 &quot; )</td>
<td>+++</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Green barley sap</td>
<td>++</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Barley chloroplasts</td>
<td>++</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

* In comparison with Table II.

Under the conditions of these experiments, serine was the major end-product in vivo, but it was not formed at all in vitro. The percentage of the total activity in each carbon of serine formed in vivo from glycolic-2-C¹⁴ acid is given in Table II. It has been shown for animals that a biological pathway for serine synthesis is from glycine whereby the $\alpha$-carbon of glycine becomes the $\alpha$- and $\beta$-carbons of serine (2). In the present experiment, $\alpha$-labeled glycolic acid was vacuum-infiltrated into barley or wheat leaves to yield serine labeled in both the $\alpha$- and $\beta$-carbons. The pathway for the formation of serine from glycolic acid is presumably through the intermediates, glyoxylic acid and glycine. The lower activity in the $\beta$-carbon of serine may be due to inactive glycine in the leaf or it may indicate the functioning of some other pathway.

A third major end-product of glycolic acid oxidation in vitro was not identified and is listed as an unknown in Table I. A large part of this substance could be precipitated from an 85 per cent alcohol solution which
was often used to stop the reaction at the end of a run. In 50 to 60 per cent alcohol it was soluble and could be placed on paper chromatograms along with the rest of the plant extract. This substance did not move in the phenol-water solvent used to develop the chromatograms and moved only as a short streak in the butanol-propionic acid-water solvent. It gave a positive ninhydrin color test on the chromatograms. After the unknown was eluted from the paper with water or precipitated by alcohol from the original mixture, it was hydrolyzed in 1 N HCl at 120° for 25 hours. All of the activity was recovered in glycine. When C\textsuperscript{14}-labeled glycine was incubated with green barley sap under the same conditions which converted glycolic acid to this unknown, there was little utilization of the glycine and the unknown was not formed.

Etiolated Pawnee wheat leaves and leaf sap utilized glycolic acid as does the green sap but at a much slower rate (4). The more even distribution

| TABLE II
<table>
<thead>
<tr>
<th>Serine from Oxidation by Plants of Glycolic-2-C\textsuperscript{14} Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant material, in vivo</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Green wheat leaves</td>
</tr>
<tr>
<td>Etiolated wheat leaves</td>
</tr>
<tr>
<td>Green barley leaves</td>
</tr>
</tbody>
</table>

of the C\textsuperscript{14} in the α- and β-carbons of serine from etiolated leaves would indicate less dilution by inactive glycine in these starved etiolated leaves.

**DISCUSSION**

These results indicate a direct pathway for the metabolism of glycolic acid to glycine and serine in higher plants. The 2-carbon chain of glycolic acid apparently goes unchanged to glycine and to the carboxy and α-carbons of serine, while the β-carbon of serine is formed chiefly from the original α-carbon of glycolic acid. Previous studies on the oxidation of glycolic acid (1, 10) have been concerned with the isolated enzyme, glycolic acid oxidase, which catalyzes the oxidation of glycolic acid to glyoxylic acid, followed by non-enzymatic oxidation of glyoxylic acid by H\textsubscript{2}O\textsubscript{2} to formic acid and carbon dioxide. The results reported here for the crude sap show further utilization of the glyoxylic acid by plant enzymes.

In this paper only the two major products from glycolic acid utilization by wheat and barley leaves are discussed. In experiments *in vivo* with glycolic acid a great number of other compounds slowly become labeled.
with C\textsuperscript{14}, but for experiments of a few minutes duration glycine and serine are the only major products. From experiments \textit{in vivo} which lasted an hour, several other products were present on the chromatograms, but none of them contained more than a small percentage of the activity found in the glycine and serine products. Recently it has been shown that glyoxylic acid is oxidized to oxalic acid by an enzyme in tobacco leaves (11). In the experiments reported here with barley and wheat leaves, oxalic acid was not a major product, but it could have been present in the longer experiments \textit{in vivo} as one of the unidentified products formed in small amounts.

Several functions for glycolic acid in plants have recently been proposed. The oxidation of glycolic acid to glyoxylic acid is a reversible system capable of functioning as a terminal oxidase in plants (10). Glycolic acid has been shown to be closely associated with the carbon cycle in photosynthesis (12). The data presented here indicate that it serves as a carbon source for glycine and serine synthesis and thus probably functions between a 2-carbon fragment of the carbon cycle in photosynthesis and these amino acids.

With the alga \textit{Scenedesmus} it has been shown that glycolic acid can serve as a carbon source for the \( \alpha \) and \( \beta \)-carbon atoms of glyceric acid (12). Equal labeling was found in these 2 carbons of glyceric acid from unequally labeled glycolic acid. This was interpreted to indicate that glycolic acid could serve as a source for a symmetrical 2-carbon compound needed in photosynthesis for the initial CO\textsubscript{2} fixation step which forms glyceric acid. Our data suggest also that part of the glyceric acid formed from glycolic acid might actually arise from the metabolic utilization of serine, which is the major and rapidly formed product from glycolic acid \textit{in vivo} and which also is about equally labeled in the \( \alpha \)- and \( \beta \)-carbons.

The source of the amino group for glycine and serine has not been investigated, although transamination between glyoxylic acid and other amino acids would be expected. This transamination must occur in isolated and twice washed barley chloroplast fragments, since no amino acids or other nitrogen source was added in the experiments.

**SUMMARY**

In barley and wheat leaves the major products formed from glycolic acid are glycine, serine, and an unknown. The carbon atoms of the glycolic acid become the corresponding ones in glycine and the carboxy and \( \alpha \)-carbons of serine. The \( \beta \)-carbon of serine is formed from the \( \alpha \)-carbon of glycolic acid.

The authors are indebted to Dr. B. M. Tolbert for the C\textsuperscript{14} labeled glycolic acid which was purchased from the Isotopes Division of the Atomic Energy Commission and for samples of C\textsuperscript{14}-labeled glycine and serine.
654 PRODUCTS FORMED FROM GLYCOLIC ACID

BIBLIOGRAPHY

GLYCOLIC ACID IN PLANTS
N. E. Tolbert and Marjorie S. Cohan


Access the most updated version of this article at http://www.jbc.org/content/204/2/649.citation

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/204/2/649.citation.full.html#ref-list-1