Oxidation of Glycine via the Respiratory Chain in Mitochondria Prepared from Different Parts of Spinach

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ABSTRACT

Mitochondria were prepared from roots, stalks, leaves, and leaf veins of spinach. The mitochondrial preparations were examined for their ability to oxidize glycine via the respiratory chain. It is shown that the glycine-oxidizing capacity is restricted to photosynthetically active tissue. The activity is present in mitochondria from the green parts of the leaves, but not in mitochondria from roots, stalks, or leaf veins.

The metabolic pathway of photorespiration involves chloroplasts, peroxisomes, and mitochondria. The reactions taking place in mitochondria are generally agreed to be the conversion of glycine to serine, NH₃, and CO₂ by the combined action of glycine decarboxylase and serine hydroxymethyl transferase (5). The decarboxylation yields NADH (3), reoxidation of which can be coupled to the respiratory chain (2, 7, 11, 12) or to endogenous malate dehydrogenase (11, 12).

Glycine decarboxylase activity has been shown to be present in mitochondria from leaves of several C₃ species (8, 12) and in bundle sheath cells of some C₄ species (NAD-malic enzyme type) (12). No activity was present in potato mitochondria (Solanum tuberosum), mitochondria from etiolated mung bean hypocotyls (Phaseolus aureus) (8), or mitochondria from maize leaves (Zea mays) (8, 12).

It was our purpose to examine the capacity to oxidize glycine via the respiratory chain of mitochondria prepared from different parts of the same plant, including different parts of the leaf.

MATERIALS AND METHODS

Plant Material. Spinach (Spinacia oleracea L. var. Viking 11) was grown at 18 C under artificial light with a light period of 12 h, in nutrient solution according to Siegenthaler and Depéry (10) except that the Fe-concentration was doubled. Different parts of the plant were used for preparation of mitochondria. Roots and stalks were cut off and rinsed in distilled H₂O. Leaves were deribbed (midvein and some of the smaller veins were removed). As much leaf material as possible was then removed from the veins with a razor blade.

Preparation of Mitochondria. The mitochondria were prepared as described previously (4) by a preparation procedure using differential centrifugation and phase partition. The homogenization time was extended to 5 s to increase the recovery. The low speed centrifugation was extended to 2 min in the preparations from roots, stalks, and leaf veins as the pellets otherwise became soft. The phase partition step was omitted for leaf veins as the veins were difficult to obtain in sufficient quantity.

Mitochondrial Assays. Respiration was measured at 25 C with a Hansatech O₂ electrode (Norfolk, England) in 0.4 ml respiration medium (0.3 M sucrose, 10 mM KCl, 5 mM MgCl₂, 0.1% (w/v) defatted BSA, 10 mM K-phosphate (pH 7.2) with 0.1-0.4 mM protein. Substrate concentrations were 10 mM for succinate, malate, and glycine, and 1 mM for NADH.

The electrode was calibrated against air-saturated water, which O₂ concentration was taken as 250 μM. ADP/O and RC were measured as described by Estabrook (3).

The intactness of the inner and outer membranes was estimated from the latency of, respectively, NAD-isocitrate dehydrogenase and succinate-Cyt c oxidoreductase activities (4). Substrate-K₅Fe(CN)₆ oxidoreductase activities were measured at 420 nm in respiration medium in the presence of 1 mM K₅Fe(CN)₆, 0.2 mM ATP, 10 mM substrate, and 0.1 mM ADP. The reactions were started with the addition of substrate and ADP. The measurements were performed at room temperature (about 22 C) under N₂, since inhibitors of Cyt oxidase also affect the activities of glycine decarboxylase and serine hydroxymethyl transferase (5, 11).

RESULTS AND DISCUSSION

Glycine decarboxylase activity in plant mitochondria is a very fragile property demanding intact inner membranes for activity (12). This criterion was met by all of the preparations used in this investigation (Table I). The preparations from leaves, leaf veins, and stalks showed relatively good RC (Fig. 1). It is therefore reasonable to assume that the mitochondria were sufficiently intact to maintain glycine decarboxylase activity. The intactness of the outer membrane of leaf mitochondria was, however, significantly lower than reported earlier (4). This probably depends on the longer homogenization time used in this study.

Substrate-dependent respiration of mitochondria prepared from different parts of spinach is shown in Figure 1. NADH, succinate, and malate were oxidized by all mitochondria tested, whereas glycine was oxidized rapidly only by leaf mitochondria. The mitochondria from stalks and leaf veins showed slow O₂ uptake.

Table 1. Intactness of Mitochondrial Membranes

<table>
<thead>
<tr>
<th>Material</th>
<th>Inner Membrane#</th>
<th>Outer Membrane#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>89 ± 2</td>
<td>79 ± 9</td>
</tr>
<tr>
<td>Leaf veins</td>
<td>90 ± 6</td>
<td>88 ± 6</td>
</tr>
<tr>
<td>Stalks</td>
<td>90 ± 4</td>
<td>86 ± 7</td>
</tr>
<tr>
<td>Roots</td>
<td>93 ± 2</td>
<td>67 ± 13</td>
</tr>
</tbody>
</table>

# Based on NAD-isocitrate dehydrogenase activity in isotonic medium with and without 0.02% Triton X-100.

# Based on succinate-Cyt c oxidoreductase activity in isotonic and hypotonic medium.

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with glycine, and ADP had only a small effect on the rate. The leaf veins could not be totally freed from green leaf material. This green material could account for the glycine-oxidizing activity found in the mitochondrial preparations from leaf veins. The background respiration rate in root mitochondria was not increased by the addition of glycine and ADP.

Table II shows the substrate-K₃Fe(CN)₆ oxidoreductase activities measured under N₂. All preparations with high activities were succinate as substrate. With glycine as substrate neither root nor leaf vein mitochondria had any detectable activity. Only leaf mitochondria showed a high glycine-K₃Fe(CN)₆ oxidoreductase activity. The activity of stalk mitochondria was low and varied much between preparations. The light-facing side of the stalks have more or less developed green wings. Mitochondria from these green cells are probably responsible for these low and variable activities.

The respiration by root mitochondria was not affected by glycine (Fig. 1) and no glycine-K₃Fe(CN)₆ oxidoreductase activity could be detected (Table II). We conclude that glycine oxidation via the respiratory chain is absent in spinach root mitochondria. The activities found in stalk and leaf vein mitochondria probably originated from the green cells, present in the starting material. We therefore assume that these activities are absent also in mitochondria from stalks and leaf veins. The results show that the ability of spinach mitochondria to oxidize glycine via the respiratory chain is restricted to mitochondria from green cells, which

Fig. 1. Oxidation of NADH, succinate, malate, and glycine by mitochondria prepared from leaves, leaf veins, stalks, and roots of spinach. Addition of substrate (both) and ADP (↓) is indicated. Numbers on the traces refer to nmol O₂ consumed per mg protein per min. Horizontal bar indicates 1 min.
**GLYCINE OXIDATION IN SPINACH**

Table II. Substrate-\(K_2Fe(CN)_6\) Oxidoreductase Activities Measured under \(N_2\)

<table>
<thead>
<tr>
<th>Mitochondria from</th>
<th>Substrate</th>
<th>(nmol K_2Fe(CN)_6) reduced per mg protein per min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>Succinate</td>
<td>169 ± 25</td>
</tr>
<tr>
<td></td>
<td>Glycine</td>
<td>85 ± 30</td>
</tr>
<tr>
<td>Leaf veins</td>
<td>Succinate</td>
<td>78 ± 10</td>
</tr>
<tr>
<td></td>
<td>Glycine</td>
<td>&lt;3(^a)</td>
</tr>
<tr>
<td>Stalks</td>
<td>Succinate</td>
<td>152 ± 25</td>
</tr>
<tr>
<td></td>
<td>Glycine</td>
<td>9 ± 10</td>
</tr>
<tr>
<td>Roots</td>
<td>Succinate</td>
<td>123 ± 33</td>
</tr>
<tr>
<td></td>
<td>Glycine</td>
<td>&lt;3(^a)</td>
</tr>
</tbody>
</table>

\(^a\) Approximate limit for detection.

Means cells having photosynthesis and photorespiration.

Moore et al. (7) observed that small amounts of oxaloacetate showed a transient inhibition on O\(_2\) consumption with glycine as substrate. Oxaloacetate was also shown to stimulate \(^{14}\text{CO}_2\) release from \(^{14}\text{C}\)glycine. This shows that the two systems for NAD regeneration in glycine decarboxylation can alternate. Our results indicate that glycine decarboxylase is absent in root, stalk, and leaf vein mitochondria from spinach.

Glycine decarboxylase activity has been reported for nonphotosynthetic tissue, for example, in mitochondria from etiolated mung bean hypocotyls (7) and tobacco root mitochondria (9). The measured activities are very low and not detectable with the methods used in this study. The metabolic significance of these low activities remains to be elucidated.

The results obtained in this study show an unequal distribution of enzyme activity between different parts of the leaf. Metabolic differences between different types of cells in a leaf are probably a common feature, are well known from C\(_4\) plants, and have also been demonstrated in C\(_3\) plants. Black (1) showed differences in enzyme distribution between the epidermis and the rest of the leaf. Most preparations from plants start from a mixed population of cell types. This heterogeneity should be considered in the study of compartmentation of metabolism.

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**LITERATURE CITED**


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