Activity of Mitochondrial Preparations Obtained from Faris Sweet Lemon Fruit

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Active mitochondrial preparations have been obtained from a wide variety of plants (1) but apparently have not been reported for citrus. Some of the difficulties which may have interfered with citrus fruit preparations are believed to be the highly acid cell sap, the low concentration of proteins, and the high concentration of pectins.

By modifying the usual methods of obtaining active mitochondrial preparations (1, 5), it was possible to obtain preparations of citrus fruit which oxidized citric acid cycle intermediates, esterified them, and formed and accumulated some organic acids.

Materials and Methods

Young Faris sweet lemons (Citrus limon Linn. Burmann), about 4 cm in diameter, were picked and cooled to 1° for 2 to 3 hours before extraction of the mitochondria. About 200 g of cold, peeled fruit were grinded in 300 ml of cold 0.6 M sucrose solution containing 0.25 M Tris buffer adjusted to pH 7.8 with KOH. It was necessary to grate the fruit directly in the buffer to neutralize immediately the acids in the cell sap. During the grating procedure, the pH of the buffer was determined with pH indicator paper occasionally, and was adjusted with 5% KOH in 0.6 M sucrose solution.

Although this was the preferred method, it was also possible to infiltrate cold buffer into the chopped fruit at a reduced pressure (10-15 cm Hg) in an
ice bath for 10 minutes and then grind in a Waring blender (500 ml container with screw cap) completely filled with buffer and closed to exclude air. Three 1-minute grinding periods were alternated with two 2-minute cooling periods to prevent overheating the homogenate. When the latter procedure for extraction was followed, it was necessary to predetermine the approximate amount of KOH required to obtain the desired final pH, and to add this amount before grinding.

The cold homogenate was strained through cheesecloth, centrifuged for 15 minutes at 2000 × g, and then for 15 minutes at 18,000 × g. The pellet obtained from the second centrifugation was washed with 35 ml of 0.5 M sucrose and 0.05 M Tris solution adjusted to pH 7.2, and resedimented for 15 minutes at 18,000 × g. The pellet was then resuspended in 5 ml buffer again. The suspension generally had a nitrogen content of 500 to 900 μg/ml.

A comparison of extraction of mitochondria with and without the inclusion of ethylenediaminetetra-acetic acid in the extracting buffer (5) showed that greater activity of the preparations was obtained when the chelating agent was omitted.

A Beckman oxygen electrode was used to determine the need for various factors for subsequent respirometer experiments. The factors used in the experiments were, in mmole: ADP, 1.0; CoA, 0.06; cytochrome c, 0.002; DNP, 1.0; FAD, 1.0; glucose, 50; Pi, 40; Mg++, 5; substrate, 30; TPP, 0.1; Tris, 75.

Oxygen uptake experiments were run at 25° in a standard Warburg respirometer. Each flask contained 2.8 ml of reaction mixture, including 0.5 ml of mitochondrial suspension. The pH was 7.1 to 7.3.

Oxidation and phosphorylation of citric, α-ketoglutaric, succinic, and malic acids were determined at different molarities of sucrose (0.25-0.75 M) and at different pH values (6.0-9.5). Phosphorylation determinations were made by measuring P1 uptake on aliquots of the reaction mixtures inactivated with 1 ml of 10% trichloroacetic acid. P1 was determined colorimetrically with reduced phosphomolybdate complex.

Paper chromatograms of the organic acids were developed in isomyl alcohol and 5 M formic acid (1:1) (1,6). Reaction mixtures were deproteinized by acidifying to pH 2.0 with 0.5 N HCl and warming in a water bath at 70° for 2 minutes. After centrifugation, 25 to 50 μl of the supernatant fraction were applied to Whatman No. 1 paper. Following development for 10 to 12 hours, chromatograms were air dried and then heated in an autoclave at 60 to 70° for 2 minutes to drive off interfering amounts of formic acid. The papers were sprayed with bromphenol blue (0.05% in 95% ethanol) to reveal the organic acids (2). Identifications were made by comparisons with standards.

When C14-labeled organic acids were used, the procedures were the same except that the activity was located both by scanning the chromatograms with a Vanguard 880 automatic scanner and by making radioautographs.

Citrate-1,5-C14 (1.09 mc/mmmole, Tracerlab, Inc.) was diluted with 0.3 M unlabeled citrate to give 5.2 × 106 cpm in 0.1 ml; succinate-1,4-C14 (3.55 mc/mmmole, New England Nuclear Corporation) was mixed with 0.3 M unlabeled succinate to give 5.6 × 106 cpm in 0.1 ml; and pyruvate-3-C14 (3.32 mc/mmmole, New England Nuclear Corporation) was mixed with 0.3 M unlabeled pyruvate to give 8.6 × 106 cpm in 0.1 ml.

**Results**

Mitochondrial preparations of Farris sweet lemons were found to oxidize citrate, α-ketoglutarate, succinate, malate and pyruvate (Table I). The cycle was inhibited by cyanide (5 × 10⁻³ M) and by malonate (1.5 × 10⁻³ M). Although measurements of relative inhibition by arsenite and fluoroacetate are not presented, these substances were found to inhibit O2 uptake by the lemon mitochondria also. The oxidation was coupled to phosphorylation, giving relatively high P/O ratios (Table I).

Intermediate rates of oxidation were obtained with citrate and α-ketoglutarate as substrates, the greatest rate was with succinate, and the lowest rates were with malate and pyruvate. Succinate also differed

**Table I. The Effect of Malonate and Cyanide on the O2 Uptake and P/O Ratio of Mitochondrial Preparations of Farris Sweet Lemon**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Control</th>
<th>Malonate (1.5 × 10⁻⁵ M)</th>
<th>Cyanide (5 × 10⁻⁵ M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μl O₂</td>
<td>P/O</td>
<td>μl O₂</td>
</tr>
<tr>
<td>Control</td>
<td>12.4</td>
<td>1.9</td>
<td>2.1</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>21.5</td>
<td>...</td>
<td>20.2</td>
</tr>
<tr>
<td>Citrate</td>
<td>226.5</td>
<td>2.3</td>
<td>193.3</td>
</tr>
<tr>
<td>α-Ketoglutarate</td>
<td>256.3</td>
<td>2.3</td>
<td>169.9</td>
</tr>
<tr>
<td>Succinate</td>
<td>318.1</td>
<td>1.6</td>
<td>198.2</td>
</tr>
<tr>
<td>Malate</td>
<td>111.3</td>
<td>2.2</td>
<td>114.0</td>
</tr>
</tbody>
</table>

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from the other substrates in that maximum O₂ uptake was achieved with only the addition of ADP and P₃.

Reaction Products. The organic acids identified in the chromatograms were citric, malic, α-ketoglutaric, succinic, and fumaric. Citric acid was less abundant than malic acid in the Faris sweet lemon.

In the experiments with citrate-1, 5-C¹⁴ as a substrate (fig 1, chromatograms 1–4), an appreciable amount of label was found in α-ketoglutarate after 60 minutes (chromatogram 2), and additionally in malate after 120 minutes (chromatogram 3). Where 1.5 × 10⁻⁵ M malonate was added, malate was not detected, but some succinate accumulated in addition to α-ketoglutarate (chromatogram 4).

In the experiments with labeled succinate-1, 4-C¹⁴ as a substrate (fig 1, chromatograms 5–8), malate and fumarate were found after 60 minutes (chromatogram 6), and additionally citrate after 120 minutes (chromatogram 7). On the addition of 1.5 × 10⁻⁵ M malonate, fumarate and citrate were not detectable while malate was present to a very limited degree (chromatogram 8).

Pyruvate-3-C¹⁴ together with 2 μmoles of malate as a sparker acid resulted in the formation of labeled citrate, malate, and α-ketoglutarate (fig 1, chromatogram 9). The incorporation of the C¹⁴ label in various acids of the tricarboxylic acid cycle indicates that the TCA cycle is operative. Unknown peaks near the origin in the various chromatograms are possibly amino acids, as inferred from related investigations.

Discussion

Part of the success in obtaining active mitochondrial preparations from citrus fruit is attributed to the use of the Faris sweet lemon in developing a method. The low acidity of this variety, which is believed to be a mutant of a commercial sour variety, minimized the inactivation which can be caused by a low pH. By use of the described procedure, however, it has also been possible to obtain active preparations from sour varieties of lemons.

The Faris sweet lemon may offer a unique opportunity for studying organic acid metabolism in citrus because of its failure to accumulate citric acid in the manner typical of most citrus. Mature sour lemons accumulate over 4 % acid, in contrast with only a fraction of 1 % in the Faris (3). In the absence of a high citric acid content in Faris sweet lemons, malic acid achieves more prominence. Whether the Faris lemon has a block in the rapid formation of citric acid or whether it metabolizes this intermediate too readily for accumulation is not known. Another possibility which might account for the low acidity in this variety lies in the mechanism regulating the movement of organic acids from the cytoplasm into the vacuole. Also, Young

![Fig. 1. Scanner records of chromatograms after incubation of labeled substrates with mitochondrial preparations. Chromatograms 1 to 4, citrate-1, 5-C¹⁴; 1, zero time; 2, 60 minutes; 3, 120 minutes; 4, 120 minutes with 1.5 × 10⁻⁵ M malonate. Chromatograms 5 to 8, succinate-1, 4-C¹⁴; 5, zero time; 6, 60 minutes; 7, 120 minutes; 8, 120 minutes with 1.5 × 10⁻⁵ M malonate. Chromatogram 9, pyruvate-3-C¹⁴ with malate sparker, 120 minutes. Peaks to the left of application points (AP) marked by arrows are labeled carbonate reference marks applied after developing the chromatograms. C, citrate; Kg, α-ketoglutarate; M, malate; S, succinate; F, fumarate.](image-url)
and Biale (8) and Huffaker and Wallace (4) have shown that citrus fruit will fix CO₂ in the organic acids and this may be an area in which the sweet and sour lemons differ.

**Summary**

Active mitochondrial preparations were obtained from the peel of young Faris sweet lemons. The high buffer capacity of the grinding solution and the maintenance of a slightly alkaline reaction while grinding the tissues directly in the solution obviated inactivation of the mitochondria usually associated with a low pH.

Citric acid occurred in a lower concentration than malic acid in the Faris sweet lemon. Oxidative phosphorylation was obtained with citrate, α-ketoglutarate, succinate, and malate as substrates. When citrate-1, 5-C¹⁴ was used as a substrate, labeled α-ketoglutarate was found after 60 minutes and labeled malate after 120 minutes. When succinate-1, 4-C¹⁴ was used as a substrate, malate and fumarate were found after 60 minutes and also citrate after 120 minutes. When pyruvate-3-C¹⁴ was used as a substrate together with a sparker acid, citrate, α-ketoglutarate, and malate were found after 120 minutes. These findings, together with the inhibitory effects of cyanide and malonate, indicated the citric acid cycle was operative in this variety of lemon.

**Literature Cited**


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**Effect of Light Quality, Light Intensity and Temperature on Pigment Accumulation in Barley Seedlings**

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**Introduction**

The first action spectrum for chlorophyll accumulation was obtained by Schmidt (Smith and Young, 19) using etiolated corn seedlings. His results indicated 3 peaks of effectiveness at 640, 567, and 450 μμ. Since the early work of Schmidt there have been a number of reports concerning the effect of wavelength on chlorophyll accumulation and on the conversion of protochlorophyll to chlorophyll. Differences in the action spectra for these 2 phenomena (19) indicate that they should be considered independently, and only the former is pertinent to the work reported in this paper.

Several reports indicate that the red region is most effective in chlorophyll accumulation (12, 13, 19), while others report the blue to be most effective (1, 5). The same disparity exists for carotenoid accumulation (14, 18, 19). In addition Kakhnovitch (7) points out that wavelength has no differential effect on accumulation if the incident energy is greater than 20,000 ergs/cm² per second.

The initial production of chlorophyll in etiolated plants is directly proportional to light intensity at relatively low incident energy (19). This relationship is not extended over long time studies or at high intensity illumination (16, 19). In long-term experiments the determination of the most effective in-

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