Sucrose Density Gradient Distribution of Mitochondrial Protein
and Enzymes from Preclimacteric and Climacteric Pears

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Summary. Mitochondrial fractions isolated from pears (Pyrus communis L.) at the climacteric minimum and peak were subjected to sucrose density gradient centrifugation. The distribution of protein and specific activities of 3 enzymes from this mitochondrial fraction were investigated.

Cytochrome oxidase specific activity remained associated with the particulate fraction and increased slightly during the period in which respiration of the whole fruit reached its climacteric peak. Catalase and acid phosphatase specific activity was associated with both the particulate and the least dense region of the gradient and decreased with post-harvest ripening.

Evidence for several differences between the subcellular behavior of catalase and acid phosphatase from pear tissue compared to their counterparts isolated from mammalian cells is discussed. A general shift of maximum specific enzymic activities and protein distribution to lighter regions of the density gradient occurs with ripening, suggestive of diminution in size or density of intracellular particles.

Several studies relating mitochondrial activity with the climacteric sequence in ripening fruit have been discussed by Biale (3) and Hulme (11). A recent report (12) indicates that the respiratory activity of mitochondria remains high, well past the climacteric peak. Bain and Mercer (1) have further shown by electron microscopy that mitochondrial structures remain virtually unaltered through the ripening period.

Nonetheless, the stress of senescence may be expected to produce some cytoplasmic adjustments as in the case of placental cells of ripening tomatoes (7). In a preceding study (17), a decrease in yield of mitochondria was noted coincident with the climacteric peak and immediate post-climacteric phase of ripening pears.

Since the particles of the mitochondrial fraction are known to play an important role in intracellular metabolism, a study on the effect of ripening on several enzymes from these organelles appeared to be of value. The sucrose density gradient centrifugation technique was utilized to further characterize the mitochondrial fraction.

Enzymes, assayed in this investigation, were chosen because each represents a specific particle type usually found in the mitochondrial fraction. Cytochrome oxidase is associated with the mitochondria, particles which contain cristae and mediate oxidative phosphorylations. Catalase is localized in the uricase-containing particle which appears to be smaller than mitochondria and contains no cristae (20). Acid phosphatase is thought to be localized in the lysosome particles of mammalian cells which are somewhat similar in size and appearance to the uricase-containing particles (2).

Thus, the purpose of this investigation was the discernment of possible intracellular changes in pears due to ripening as reflected by the protein and enzyme distribution within the mitochondrial fraction.

Materials and Methods

Mature (preclimacteric) Bartlett pears used for experimentation were freshly picked and stored at 0° for approximately 3 days. Fruit from the same lot were placed in respiration jars at 20° and respiration was measured over a period of 10 days by the method of Claypool and Keefer (6). At the climacteric minimum and peak, representative samples of pears were taken for mitochondrial fraction isolation and the determination of its protein and enzyme distribution.

Mitochondrial Isolation. Pears were peeled and finely grated. One hundred g wet weight of tissue
was combined with 50 ml of medium A (0.25 M sucrose, 0.01 M cysteine and 0.02 M Tris, pH 7.5) and homogenized by pressing through a colander. A predetermined volume (4.5 ml) of 1 M Tris solution was also added during homogenization to neutralize the endogenous acidity of the fruit. The mash was squeezed through 2 layers of cheesecloth and the resulting filtrate was diluted to 240 ml with medium A and centrifuged at 770 × g for 15 minutes. The supernatant fraction was decanted, filtered through 2 layers of cheesecloth and recentrifuged at 18,000 × g for 15 minutes. The mitochondrial pellets were combined, resuspended in 80 ml of medium A and recentrifuged at 18,000 × g for 15 minutes.

The once washed mitochondrial pellets were homogenously resuspended in 5.0 ml of medium B (0.25 M sucrose, 0.05 M Tris, pH 7.5 and 1 × 10⁻⁴ M EDTA). One ml aliquots were layered over sucrose density gradients (S.D.G.) in Spinco SW-25 rotor tubes and centrifuged at 34,500 × g for 60 minutes. Linear sucrose density gradients ranging from 8.5 to 65 % were prepared by mixing the 8.5 % sucrose medium into the chamber containing the 65 % sucrose medium as the gradient was being formed by a method similar to that of Britten and Roberts (4).

After centrifugation, a pinhole was made at the bottom of the S.D.G. tube and the contents carefully subdivided into 10 approximately equal volume fractions of decreasing densities. All isolation steps were carried out at 0°. The S.D.G. fractions were frozen until assayed.

Assay Procedures. Cytochrome oxidase activity was determined spectrophotometrically (19), and catalase activity was measured by the potassium permanganate titration method (5). The method of Gianetto and de Duve (9) was used to determine acid phosphatase activity utilizing β-glycerophosphate at pH 5 as a substrate. Protein was determined by the technique of Lowry et al. (13) with crystallized bovine serum albumin as a standard.

Results and Discussion

The Bartlett pears (Pyrus communis L.) used in these experiments produced the typical climacteric respiration curve (fig 1) as indicated by the increased rate of CO₂ evolution with ripening at 20°C. Mitochondrial fractions for protein and enzyme distribution studies were isolated from pears at the climacteric minimum and peak.

The S.D.G. protein distribution of pear mitochondrial fractions (fig 2) shows that the bulk of the mitochondrial protein from pre-climacteric pears is in the denser region of the gradient and has a higher and sharper distribution peak compared to mitochondria from climacteric fruit. Similar S.D.G. distribution curves have been observed for ripening avocados (16) and Bing cherries (18).

The change in distribution pattern suggests that the mitochondrial components may diminish in size and density as a result of ripening. Greater ease of fragmentation of the mitochondria during homogenization of the riper fruit is another possibility. Mitochondrial fragmentation has previously been noted with both plant (10) and mammalian cells (20).

The distribution peak of cytochrome oxidase specific activity corresponds closely with that of protein distribution for the pear mitochondrial fraction (fig 3). A small increase in cytochrome oxidase specific activity occurs as a result of fruit ripening (table 1) paralleling the increased respiration of the fruit during the ripening period (fig 1).

In contrast to the single peak of the S.D.G. dis-
Fig. 3. Pear mitochondrial cytochrome oxidase specific activity distribution after sucrose density gradient centrifugation. The continuous and dashed lines represent isolations from pears at the climacteric minimum and peak, respectively. Specific activity is expressed as units per second per mg protein.

Fig. 4. Pear mitochondrial catalase specific activity distribution after sucrose density gradient centrifugation. The continuous and dashed lines represent isolations from pears at the climacteric minimum and peak, respectively. Specific activity is expressed as units per second per mg protein.

Fig. 5. Pear mitochondrial acid phosphatase specific activity distribution after sucrose density gradient centrifugation. The continuous and dashed lines represent isolation from pears at the climacteric minimum and peak, respectively. Specific activity is expressed as umoles of $P_1$ per minute per mg protein.

Table I. Specific Activities of Enzymes in the Mitochondrial Fraction Isolated from Pears

<table>
<thead>
<tr>
<th>Ripening time (Days)</th>
<th>Cytochrome oxidase* ($\times 10^3$)</th>
<th>% Change</th>
<th>Catalase* ($\times 10^4$)</th>
<th>% Change</th>
<th>Acid phosphatase** ($\times 10^3$)</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 (Climacteric) minimum</td>
<td>34.4</td>
<td>...</td>
<td>11.4</td>
<td>...</td>
<td>160</td>
<td>...</td>
</tr>
<tr>
<td>9 (Climacteric) peak</td>
<td>38.2</td>
<td>+11</td>
<td>9.3</td>
<td>−18</td>
<td>100</td>
<td>−38</td>
</tr>
</tbody>
</table>

* Cytochrome oxidase and catalase specific activity are expressed as units per second per mg protein.

** Acid phosphatase specific activity is given in umoles $P_1$ produced per minute per mg protein.

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In mammalian cells, acid phosphatase and catalase have been localized within discrete particles surrounded by rate-limiting membranes (8). Preliminary evidence indicates that the sites of these enzymes in pear cells differ somewhat from those in mammalian cells. One difference is shown by the failure of pear catalase and acid phosphatase to be released into the surrounding medium when the mitochondrial fraction is treated with 1 mm phosphate buffer at pH 2. 0, 5% deoxycholate or 65% sucrose solution (unpublished data). A release and concomitant large increase in enzyme activities is known to occur under the above conditions with mammalian cell mitochondrial fractions (14, 15). Pear mitochondrial catalase and acid phosphatase S.D.G. curves show 1 peak of specific activity corresponding to the particulate region and another peak which remains in the uppermost or least dense portion of the gradient. In contrast, catalase and acid phosphatase of mammalian cells are associated specifically with the particulate region of the S.D.G. (2).

Changes in S.D.G. distribution of mitochondrial fractions and associated enzymes in ripening fruit suggest that considerable intracellular adjustments occur during the aging of fruit tissue as recently noted by others (1, 3, 7) including the decrease in yield of mitochondrial protein (17).

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Literature Cited