Glassy State in Plant Cuticles during Growth

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Z. Naturforsch. 49c, 273-275 (1994); received December 21, 1993

Cuticles are thin extracellular layers that cover the aerial parts of all higher plants in areas lacking secondary growth. Their chemical and morphological roles as protective barriers between the plant and its environment has been reported (Holloway, 1982). Plant cuticles are heterogeneous in nature. They consist of epicuticular wax fractions and, mainly, cutin which is a highly cross-linked polyester made of hydroxy fatty acids. In animal skin, mechanical strength and molecular barrier functions are distinct than in plant and its environment has been reported (Holloway, 1982). They consist of epicuticular wax fractions and, mainly, cutin which is a highly cross-linked polyester made of hydroxy fatty acids. In animal skin, mechanical strength and molecular barrier functions are distinct than in plants and both of these properties are served by proteins. As opposed to the animal barriers, our knowledge of the molecular dynamic and thermal properties of plant cuticles is very limited (Lendzian and Kerstiens, 1991).

Table I. Quantities of waxes, hydrolyzable components (HYD) and cutin (in μg cm⁻²) isolated from tomato fruit cuticles (Lycopersicon esculentum, Mill. cv. Caruso) at various stages of maturity. Parenthesis indicate the standard deviations of three different determinations.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>41.7</th>
<th>59.6</th>
<th>Fruit diameter [mm]</th>
<th>66.5</th>
<th>82.2</th>
<th>80.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cuticle weight</td>
<td>407 (90)</td>
<td>940 (48)</td>
<td>1290 (99)</td>
<td>1516 (143)</td>
<td>1653 (38)</td>
<td></td>
</tr>
<tr>
<td>Waxes</td>
<td>19.7 (0.2)</td>
<td>18.1 (5.1)</td>
<td>46.0 (2.5)</td>
<td>44.0 (8.8)</td>
<td>61.6 (1.7)</td>
<td></td>
</tr>
<tr>
<td>Cutin</td>
<td>196.3 (5.7)</td>
<td>549.5 (19.9)</td>
<td>810.3 (8.5)</td>
<td>990.9 (14.9)</td>
<td>1125.8 (52.9)</td>
<td></td>
</tr>
<tr>
<td>HYD</td>
<td>191.0 (5.7)</td>
<td>372.4 (25.1)</td>
<td>434.5 (9.5)</td>
<td>482.0 (22.1)</td>
<td>466.0 (51.2)</td>
<td></td>
</tr>
</tbody>
</table>

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Verlag der Zeitschrift für Naturforschung, D-72072 Tübingen
0939-5075/94/0300-0273 $ 03.00/0
Temperature (°C)

Fig. 1. Warming thermograms of isolated tomato fruit cuticles at different growth stages obtained using differential scanning calorimetry. The thermograms have been normalized per weight unit of isolated cuticle. Cuticle weight per area unit (µg cm⁻²) were: a: 407, b: 940, c: 1291, d (mature green tomato): 1516 and e (ripe tomato): 1653. T_g and T_s indicate the onset of the glass transition and secondary transition temperatures, respectively.

Calorimeter at room temperature. In a typical run, the sample was cooled at 10 °C/min to -90 °C and the thermograms were recorded during heating at 10 °C/min against an empty aluminum pan placed in the reference vessel of the instrument. Second-order glass transitions were detected by a shift in power and they appear as step-like transitions (Vertucci, 1990). To obtain the final thermogram of the sample, the baseline was subtracted from the data obtained from each sample. The onset temperature of the second-order phase transition was determined as the point of intersection between the baseline with the tangent through the point of inflection of the thermal event.

Thermal signatures observed in all thermograms can be summarized as follows: a common second order phase transition temperature at -30 °C was assigned to the glass transition associated with conformational changes of the molecule main chain, i.e. the large methylene chains densely packed. An additional endothermic between 30 °C and 70 °C was also observed. It reflects the contribution of a second order transition of the polymer in addition to the influence that the waxes have on the whole polymer. This secondary transition appears in all thermograms and it is characterized by an onset temperature of 30 °C. This transition of the biopolymer probably involves conformational changes that do not require main chain movement. Near 45 °C the cuticular waxes begin to melt and the corresponding change in the heat flow gives the energy of this physical event.

Many important conclusions can be elucidated in spite of the similar shape of thermograms recorded for all different cuticles. First, the temperature range where the glass transition is extended increases from 16 °C for isolated cuticles from fruits of the first growth stage to 40 °C for isolated cuticles from ripe fruits (Fig. 1). Second, the corresponding energies per cuticle weight for the -30 °C and 30 °C polymer second order transitions are notably higher for ripe tomato cuticles compared to cuticles of other growth stages. These data suggest a dynamic picture of the tomato fruit cuticle during its development. At an early developmental stage, intracuticular waxes and phenolics free or attached to the cutin are absent (Baker et al., 1982). Thus, the segmental mobility of the chains of the polyester, which begins just at the glass transition temperature, is more cooperative because the homogeneity of the polymer matrix and, consequently, it is extended over a short temperature range. During fruit growth, the cuticle incorporates intracuticular waxes which are partially hindering the free motion of the cutin hydrocarbon chains. In addition, during ripening noticeable amounts of flavonoids appear trapped to the cuticular matrix. As a consequence of these molecular changes, the transitions become less cooperative and they require more energy to produce segmental mobility in the polymer matrix.

This is the first report that describes the existence of a glass transition temperature in isolated cuticles. This temperature has been measured in isolated cuticular membranes but this physical property must be ascribed to the amorphous cutin polymer that constitutes the framework of the plant cuticle. The presence of hydrolyzable compounds in the cutin could modify the value of this temperature. Current research is under way in order to determine the exact glass transition temperature of the cutin of plant cuticles.

This work has been supported by Dirección General de Investigación Científica y Técnica (Project PB91-0768).

