Glassy State in Plant Cuticles during Growth

Patricia Luque and Antonio Heredia

Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Málaga, 29071 Málaga, Spain

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

Cuticle, Differential Scanning Calorimetry, Glass Temperature, Phase Transition

The existence of a glassy state in isolated tomato fruit cuticles was investigated using differential scanning calorimetry. Tomato fruit cuticular membranes showed a glass transition temperature at −30 °C and an additional second order transition temperature near 30 °C. Changes in these temperatures during fruit growth were also studied.

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 epi- and intracuticular 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).

We are currently characterizing defined phase transitions in isolated cuticular membranes. As part of a comprehensive study of cuticle structure, we have been studying glass transitions of isolated tomato fruit cuticles over a wide range of temperatures during plant growth using differential scanning calorimetry (DSC).

Table I shows the weight per unit surface of area for the three different cuticular components (waxes, hydrolyzable components and cutin) during tomato fruit development. Between the immature green to ripe stages, the cutin content of the membrane increased to the extent that this polymeric matrix comprised over 70% of total weight. Significant changes were also noted in the fraction of hydrolyzable compounds (mainly phenolics and polysaccharide material). During the same period the proportion of this fraction decreased from 47% in small fruits to 28% in the ripe ones. On the other hand, the wax proportion was always the lower of the three cuticular components. It did not change significantly during fruit growth.

Figure 1 shows the warming thermograms of the five different isolated tomato fruit cuticles obtained from the corresponding fruits harvested at different development stages. All experiments were performed in a differential scanning calorimeter with computer-aided data analysis. The thermal head was cooled with liquid nitrogen using a low temperature cooling unit. All experiments followed the same protocol. Sealed aluminum pans containing between 7–10 mg per sample of each isolated tomato fruit cuticle, were loaded into the

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.

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

Reprint requests to Dr. Antonio Heredia. Telefax: 34-52-13 20 00.

Verlag der Zeitschrift für Naturforschung, D-72072 Tübingen
0939–5075/94/0300–0273 $ 03.00/0

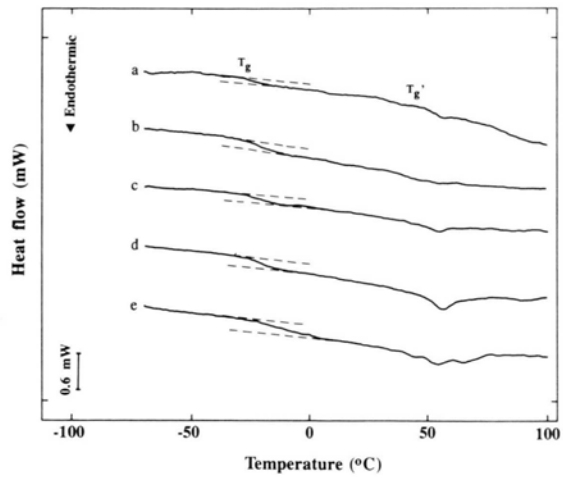

Fig. 1. Warming thermograms of isolated tomato fruit cuticles at different growth stages obtained using differential scanning calorimetry. The thermograms have been normalized per weight unit of isolated cuticle. Cuticle weight per area unit (μg cm^{-2}) were: a: 407, b: 940, c: 1291, d (mature green tomato): 1516 and e (ripe tomato): 1653. T_g and T_g' 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 substracted 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 onsent 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 adscribed 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).

Baker E. A., Bukovac M. J. and Hunt G. M. (1982), Composition of tomato fruit cuticle as related to fruit growth and development. In: The Plant Cuticle (D. F. Cutler, K. L. Alvin and C. E. Price, eds.), Academic Press, New York, pp. 33–44.

Holloway P. J. (1982), Structure and histochemistry of plant cuticular membrane: an overview. In: The Plant Cuticle (D. F. Cutler, K. L. Alvin and C. E. Price, eds.), Academic Press, New York, pp. 45–85.

Lendzian K. J. and Kerstiens G. (1991), Sorption and transport of gases and vapors in plant cuticles. Rev. Environ. Contam. Toxicol. **121,** 65–128.

Vertucci C. W. (1990), Calorimetric studies of the state of water in seed tissues. Biophys. J. **58,** 1463–1471.