Water sorption–desorption in conifer cuticles: The role of lignin

José J. Reina, Eva Domínguez and Antonio Heredia*

Departamento de Biología Molecular y Bioquímica, Grupo de Caracterización y Síntesis de Biopolímeros Vegetales, Facultad de Ciencias, Universidad de Málaga, E-29071 Málaga, Spain

*Corresponding author, e-mail: heredia@uma.es

Received 14 August 2000; revised 8 January 2001; in final form 28 February 2001

Current information on the type and amount of biopolymers present in the epidermis of conifer species is still insufficient. This work presents the detailed morphology and chemical composition of Araucaria bidwillii cuticle after selective treatments to remove the different types of biopolymers. After removal of the waxes, cutin and polar hydrolyzable components, a lignin-like fraction, which makes up 25% of the initial cuticle weight, was identified by GC-MS and infrared spectroscopy. The isolated lignin is of G type, mainly formed by guaiacyl units. This composition indicates that the conifer cuticle investigated here has similar composition to other conifer-isolated cuticles. Water sorption and desorption by the isolated cuticle and the different cuticle fractions, including lignin, were studied. The analysis of the isotherms, following distinct physicochemical models, gave useful information on the structural and physiological role of the different biopolymers present in the cuticle. Lignin fraction showed both a high water sorption and capability of retaining it in comparison to other cuticle components. Hysteresis effect on water sorption–desorption cycle and water cluster formations has also been studied, and their physiological role discussed.

Introduction

All regions of the outer epidermal walls containing the biopolymer cutin are commonly referred to as the cuticular membrane or the cuticle. The term includes both the peripheral regions formed by cutin, the cuticle proper, and those regions where the epidermal cell walls are incrusted to some degree with the cutin, the cuticular layers (Holloway 1982, Jeffree 1996).

Cuticles of higher plants are chemically heterogeneous by nature, basically consisting of a wax fraction, soluble in organic solvents, and an insoluble cuticular matrix, which forms the framework of the cuticle. This cuticular matrix is mainly formed by the biopolymer cutin, a high-molecular weight polyester composed of various inter-esterified C16 and C18 hydroxyalkanoic acids (Holloway 1982). Once all the wax and cutin components have been removed from an isolated cuticle preparation, there is usually some remaining non-lipophilic material. For many species, this residual material contains polysaccharides, proteins and other polar compounds representing the portion of the epidermal cell wall to which the cuticle was attached. From a physiological point of view, the existence of this fraction at the cuticular level, the cutinized cell wall, gives the plant cuticle an important macromolecular domain with physical and chemical capacity to interact with water and polar solutes (Domínguez and Heredia 1999).

In some species, fundamentally conifers, the presence of lignin-like material in the transition zone between cell wall and cuticle has been documented (Juniper and Jeffree 1983, Lendzian et al. 1986). Although the presence of lignin in this innermost part of the cuticular layer has been observed, a detailed study of the lignin and its physiological role at cuticular level has been conducted only for Norway spruce cuticle (Picea abies; Kögel-Knabner et al. 1994).

In this work, the chemical characterization, with special emphasis on the lignin content of the cuticle of the conifer Araucaria bidwillii Hook., is presented. In order to obtain more information about the physiological role and function of this complex cuticle, their hydrodynamic properties have also been investigated. In this sense, a complete physicochemical study of the water sorption by the cuticle and by their different lipidic and nonlipidic components has also been made.
Materials and methods
Cuticle isolation and composition

Whole leaves were obtained from Araucaria bidwillii Hook. in the central park of Málaga (Spain). Leaves 1–2 years old were picked from the corresponding stems and cuticles of A. bidwillii. They were isolated enzymatically using an aqueous mixture of 20% (w/v) pectinase (Fluka, Seelze, Germany) and 2% (w/v) cellulase (Sigma, St. Louis, MO, USA), according to the procedure described by Lendzian et al. (1986), and buffered at pH 3.6 with sodium acetate and acetic acid. After 5–7 days of incubation at 35°C in a water-bath shaker, the upper and lower (adaxial and abaxial) cuticles were recovered, washed and rinsed with abundant distilled water in order to remove residues from the cell walls. Isolated cuticles were stored at room temperature, before being used for the different experiments.

Determination of the different components of the isolated cuticles was achieved following a series of extraction-depolymerization procedures commonly used to remove cuticular waxes, to isolate the polyester cutin, and to remove the hydrolyzable polar compounds present in the plant cuticle (Riederer and Schönherr 1984). Cuticular waxes or soluble cuticular lipids were extracted by refluxing the isolated cuticles in chloroform for 24 h. This procedure yielded cuticle residue 1. This residue was then subjected to acid hydrolysis to remove polar compounds such as polysaccharides, phenols and peptides, using 6 M HCl at 110°C for 12 h. The cuticle residue 2 obtained was saponified in order to remove the cutin matrix by refluxing the solid residue in a KOH (1%, w/v) solution in methanol for 12 h. This treatment yielded cuticle residue 3, which was subjected to CuO oxidation (Chen 1992). About 10 mg of the solid sample was treated with alkaline cupric sulfate (100 mg CuSO4, 2 ml 2 M NaOH) at 180°C for 4 h using a 10 ml pressure bomb of stainless steel. For further extraction and analysis of the oxidation products, the solution was acidified to pH 3 with a 2 M HCl solution. The yield of the different residues was gravimetrically determined by weighing each residue before further treatment.

Samples obtained from the different procedures mentioned above were chemically prepared for GC analysis (Hewlett-Packard GC-MS, model 5890, Avondale, PA, USA) followed by mass spectrometry identification after chemical derivatization with N,O-(trimethylsilyl)acetamide (Sigma). Details on experimental conditions have been reported in our laboratory (Casado and Heredia 1999).

Infrared spectroscopy

Infrared (IR) spectra of samples corresponding to the cuticle residue 3 were recorded using a Perkin Elmer IR spectrophotometer (model 883, Norwalk, CT, USA) using CaF2 windows and a spectral resolution of ±0.5 cm⁻¹.

Water sorption isotherms

Water sorption on cuticle and on different cuticle residues was measured over the whole relative humidity (or water activity) range using an electrobalance, devices and procedures previously described for the study of water sorption on fruit cuticles (Luque et al. 1995) and cutinized cell walls (Dominguez and Heredia 1999).

Sample size and statistics

The weight of the cuticular components was measured with three replicates, which were further used to obtain the corresponding water sorption isotherms. Results are given as means with the standard deviation. Analytical composition (GC-MS analysis) of the different cuticular components was determined in duplicate. In all cases, the differences of the specific analytical composition of one isolate were never higher than 15%.

Results

Cuticular components and composition

The recommendations pointed out by Lendzian et al. (1986) to isolate gymnosperm cuticles, using high concentrations of hydrolytic enzymes, were successful for the conifer A. bidwillii investigated here. The enzymatically isolated cuticle of A. bidwillii showed a high degree of structural integrity, similar to that observed for other isolated conifer cuticles such as Abies alba, Pinus mugo or Picea abies (Lendzian et al. 1986). Scanning electron micrographs (microphotographs not shown) demonstrated that the isolated cuticular membranes were intact showing a well-developed architecture of the inner side of the membranes.

The weight of each cuticular component, obtained after selective extraction and degradation procedures, is given in Table 1. The amount of soluble cuticular waxes was low, and similar to those found in other conifers (Chamel et al. 1992, Kögel-Knabner et al. 1994). Similar high content of hydrolyzable components with a weight almost equivalent to the rest of the isolated cuticle was also reported. Whereas Chamel et al. (1992) named cutin as the remaining residue for A. alba cuticles, Kögel-Knabner et al. (1994) demonstrated, in isolated cuticles from P. abies needles, that the corresponding fraction obtained after acid hydrolysis contained the cutin polyester, together with a lignin-like residue, which represented about 17% of the initial cuticle weight. For A. bidwillii cuticle, we have found and identified a lignin fraction, which represents more than 25% of the initial cuticle weight (Table 1).

GC-MS analysis of the different fractions revealed as main components of the soluble cuticular waxes the sec-

Table 1. Cuticular composition of the isolated cuticle of Araucaria bidwillii. The weight of the different fractions is given in % (w/w). Means ± SD (n = 3).

<table>
<thead>
<tr>
<th>Cuticle fraction</th>
<th>Weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waxes</td>
<td>4.2 ± 0.1</td>
</tr>
<tr>
<td>Cutin</td>
<td>26.8 ± 2.5</td>
</tr>
<tr>
<td>Hydrolyzable compounds</td>
<td>42.3 ± 0.6</td>
</tr>
<tr>
<td>Lignin</td>
<td>26.7 ± 2.6</td>
</tr>
</tbody>
</table>
ondary alcohol nonacosan-10-ol (65% of wax sample) together with the n-alkane of 29 carbon atoms, n-nonacosane (16%) and other minor components (19%, mainly fatty acids and diols). The cutin polyester was mainly composed by the C16 dihydroxy fatty acid, 9,16-dihydroxyhexadecanoic acid and their corresponding 8,16- and 10,16-dihydroxy positional isomers (52.5% of cutin sample) together 16-hydroxyhexadecanoic acid (12%) and other hydroxy fatty acids and unidentified compounds (35.5%). These lipidic components have been identified in the cutin of *P. abies* cuticle and other conifer cuticles (Matzke and Riederer 1992).

CuO oxidation is commonly used for the characterization of lignin in plant materials and geochemical samples, and it was used to characterize the lignin fraction of *P. abies* cuticle (Kögel-Knabner et al. 1994). The chemical composition of the lignin fraction present in *A. bidwillii* cuticular membrane was in agreement with the monomeric composition of spruce lignin, and from the oxidation products identified as vanillin (70.5% of residue), vanillic acid (14%), acetovanillone (11%) and others (4.5%), we can infer that it was composed almost exclusively of *p*-coniferyl units (Higuchi 1990). The existence of a lignin of the named guaiacylpropane structure or G-type (Brett and Waldron 1996) was also confirmed after the analysis of the infrared spectrum of the remaining residue. Fig. 1 shows the infrared absorptions, between 4000 and 800 cm\(^{-1}\), of this sample which, in addition to other characteristic bands, presents absorptions at 1690 and 1150 cm\(^{-1}\) assigned to C\(/\mathrm{p}\)O stretch in conjugated \(\mathrm{p}\)-substituted benzenes and aromatic C–H in plane deformation, respectively, typical for G units (Faix 1991). Finally, the presence of lignin material in the cuticles of *A. bidwillii* leaves was confirmed after staining the corresponding leaf thin sections with acidic phloroglucinol (data not shown).

**Water sorption isotherm analysis**

Water sorption and desorption studies were developed for the *A. bidwillii* cuticle, as well as for two different mixtures of cuticle isolates containing the polar hydrolyzable components and lignin and cutin with lignin, respectively, and for the lignin fraction. Isotherm data were obtained over a wide activity range of 0.1–0.9 and at 25°C of temperature (Fig. 2). The figure shows the sorption and desorption isotherms for one sample of the different isolates. In all cases, the standard deviation of 3 experiments was lower than 5%. The sorption curves follow the expected sigmoidal shape, except for the case of the lignin residue, where an exponential shape can be clearly observed. Maxima water content (w/w) was 16% for the isolated cuticle, 17% for the residue fraction containing polar hydrolyzable components + lignin, 11% for the residue formed by cutin + lignin and 11% for the lignin fraction. All desorption curves had a similar behavior, following a straight line.

In order to analyze the isotherms, some equations were fitted. Among the different models present in the literature with adjustable parameters related to physical properties of the adsorbent, the Hailwood and Horrobin (1946) mathematical model was selected for the study of the sorption isotherms. This model has been previously applied to different biological models, including isolated cuticles and cutinized cell walls (Domínguez and Heredia 1999). The Hailwood and Horrobin model was developed from polymer solution theory, and assumes a simple type of binding site and the formation of an ideal solid solution. This equation includes only three adjustable parameters:

\[
W = K_1 \left( \frac{K_2 a_1}{1 + K_2 a_1} + \frac{K_3 a_1}{1 - K_3 a_1} \right)
\]  

(1)

The first term of the equation is related to sorption at strong binding sites in terms of one or more Langmuir isotherms. The second term describes the formation of a multilayer. In the equation, \(W\) is the weight of water adsorbed per gram of solid sample and \(a_1\) the water activity. The parameters with biological meaning are: \(K_1\), an expression of the number of strong binding sites, \(K_2\) the attraction of these sites for water,
and \( K_3 \) is related to the water activity of the solid solution.

Table 2 shows the results of the adjustable parameters after their estimation with the Hailwood and Horrobin model for the 3 more complex residues, and also for comparison of other cuticle samples. The sorption coefficients were calculated from a least square \( (r^2 > 0.99) \) using non-linear regression. These constant values permit a first estimation of the water binding sites and their affinities. Assuming the values reported for other systems, we can infer that the whole cuticle and the hydrolyzable components + lignin residues sorb water to an average number of binding sites, but with a high affinity, while cutin + lignin shows a lower number of sites, but also a high affinity for water. On the other hand, \( K_3 \) values showed that all samples had a relatively important affinity to form multilayers or water clusters.

In the case of lignin residue, the Hailwood and Horrobin equation did not fit the experimental data. Several other models were tried with identical results. The best fit was found when only the third term of the D’Arcy and Watt (1970) model was used. This mathematical model predicts the presence of strong and weak binding sites, as well as the formation of a solid solution. While the first two terms of the equation are related to sorption at strong and weak binding sites respectively, the third term postulate the formation of multilayers. This last term of the D’Arcy and Watt equation includes two adjustable parameters with thermodynamic properties:

\[
W = \frac{K_4 K_5 a_1}{1 - K_3 a_1}
\]

where \( W \) and \( a_1 \) are the same as above, and \( K_4 \) and \( K_5 \) correspond to the number and water activity of the multilayer binding sites, respectively. Values obtained for these two parameters (Table 2) indicate that lignin has a low number of multilayer sites \( (K_4 = 0.0226) \), but with an important affinity \( (K_5 = 0.9267) \). These results were similar to those found for the other fractions: low number of binding sites, but high affinity for water.

Fig. 2 also shows an important feature: water sorption and desorption isotherms are not coincidental, indicating the presence of a hysteretic process. Sorption hysteresis has been shown for many polymeric sorbents (Lüscher-Mattli and Rüegg 1982), and hysteresis requires an irreversible event to occur during sorption. This event is a swelling of the matrix that increases the number of active sites, allowing a higher sorption. This swelling alters the polymer structure, modifying the desorption process (Lüscher-Mattli and Rüegg 1982).

The surface expansion model of Giles et al. (1974) was used to analyze the desorption isotherms. This model, based on Henry’s law, postulates that the surface available for adsorption increases proportionally with the amount of solute adsorbed. Its mathematical expression is a straight line \( (W = B a_1) \), where the slope is the named expansion factor. In our case, the surface available for desorption diminishes with the amount of water desorbed, and desorption increase by \( B \) (expression of the polymer shrinking) with the water activity. \( B \) values obtained with this model were 0.162 (cuticle), 0.174 (hydrolyzable components + lignin), 0.112 (cutin + lignin) and 0.113 (lignin). These results show that in the first two cases, water is eliminated more easily than cutin + lignin and lignin fractions, indicating that the cuticle fraction composed by the hydrolyzable components are responsible for the greatest shrinking of the polymer and, in their absence, the polymer shrinking is lower.

Taking into account the prediction for the existence of water clusters in all the isolates investigated, an evaluation of the water activity range at which this association occurs was made. Clustering formation can explain some transport phenomena occurring in polymeric systems such as the

---

**Fig. 2.** Water sorption (•) and desorption (○) (expressed as weight of water per weight of the dry sample) of different *A. bidwillii* cuticle fractions as a function of the water activity at 25°C. A, isolated cuticle sample; B, residue containing hydrolyzable components + lignin; C, residue formed by cutin + lignin; and D, lignin isolate. Sorption isotherm curves in A, B and C were obtained after fitting to the Hailwood and Horrobin model (Eq. 1), whereas the sorption isotherm D was obtained after fitting to the D’Arcy and Watt model (Eq. 2). All desorption isotherms were fitted to a straight line based on Henry’s law, \( W = B a_1 \). For more details, see text.
Cuticle sample. As was expected, a straight line ($r^2$ function against water activity for the case of isolated Heredia 1999). Fig. 3 shows the plot of the clustering the two fractions hydrolyzable components ter formation. The values of water activity obtained for clustering begins at very low water activities.

Predicting multilayer binding sites, being assumed that clusters and applying the Zimm and Lundberg function, the clustering equation, $G_{11}$, is:

$$G_{11}/V_1 = -V_2[a_i/V_2/(a_i) - 1]$$

when $G_{11}/V_1$ is greater than $-1$, the solute is expected to cluster. $V_1$ is the volume fraction of the solute, $V_2$ the volume fraction of the polymer, and $a_i$ is the water activity. To estimate this function, it must be applied to a sorption isotherm equation that describes the experimental data. In order to simplify the calculation procedure, the Guggenheim–Andersen–De Boehr (GAB) isotherm was used in the clustering function (Luque et al. 1995). Although the GAB isotherm fully described the sorption data, with $r^2 = 0.99$ for all residues, it was not previously used because the coefficients are meaningless. The following equation is the mathematical expression of the GAB model:

$$W = \frac{m_0 \, cka}{(1 - ka_i)(1 - ka_1 + cka_i)}$$

where $m_0$ estimates the amount of sorbent required to form a monolayer, and $c$ and $k$ are adjustable parameters without physicochemical meaning.

Rewriting the GAB isotherm in terms of volume fractions and applying the Zimm and Lundberg function, the $G_{11}/V_1$ values can be obtained for given values of water activity (for mathematical procedure, see Domínguez and Heredia 1999). Fig. 3 shows the plot of the clustering function against water activity for the case of isolated cuticle sample. As was expected, a straight line ($r^2 > 0.99$) was obtained. At a water activity of 0.57 (57% of relative humidity), $G_{11}/V_1$ reaches a value of $-1$, predicting cluster formation. The values of water activity obtained for the two fractions hydrolyzable components + lignin and cutin + lignin were very similar (0.58 and 0.57, respectively). For lignin, cluster formation was not studied because the model applied to the whole isotherm only predicts multilayer binding sites, being assumed that clustering begins at very low water activities.

### Discussion

Cuticles isolated from leaves of *A. bidwillii* presented characteristics similar to other conifer cuticles. Their isolation needed a high concentration of hydrolytic enzymes and long time periods of incubation, a fact qualitatively correlated with the presence of lignin in the outer epidermal cell wall (Lendzian et al. 1986).

The morphology of the *A. bidwillii* cuticle and its chemical composition is quite similar to that of other conifers. Thus, the amount and composition of the two lipidic fractions present in the cuticle, the cuticular waxes and the polyester cutin, are almost the same as those in *P. abies* (Matzke and Riederer 1992, Kögel-Knabner et al. 1994). The cutin of the conifer investigated here is a typical C$_{16}$ cutin, formed by a dense amorphous network of dihydroxyhexadecanoic acids forming ester bonds.

The most abundant fraction found in the cuticle of *A. bidwillii* has a non-lipidic nature. It is constituted by a high amount of the heterogeneous fraction of polar hydrolyzable components together with the presence of lignin material (Table 1). Whereas the presence of the fraction of hydrolyzable components has been documented in other conifers (Chamel et al. 1992) and in cuticles of angiosperm species (Jeffree 1996), the presence and exact nature of lignin at the cuticular level has only been documented in *P. abies* needles (Kögel-Knabner et al. 1994). According to the description made by Tenberge (1992) on the ultrastructure of *P. abies*

### Table 2. Isotherm constants estimated for the different isolates from *Araucaria bidwillii* cuticle and other samples after the application of the Hailwood and Horrobin or the D’Arcy and Watt models (Eqs. 1 and 2, respectively) at 25°C. For the meaning of the different parameters, see text. * Luque et al. (1995); † Domínguez and Heredia (1999); HC, hydrolyzed components.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Fruit cuticle$^a$</th>
<th>Cutinized cell wall$^b$</th>
<th>Cuticle</th>
<th>HC</th>
<th>Cutin + lignin</th>
<th>Lignin</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_1$</td>
<td>0.230</td>
<td>0.050</td>
<td>0.045</td>
<td>0.047</td>
<td>0.030</td>
<td></td>
</tr>
<tr>
<td>$K_2$</td>
<td>4.86</td>
<td>7.43</td>
<td>10.43</td>
<td>12.70</td>
<td>11.18</td>
<td></td>
</tr>
<tr>
<td>$K_3$</td>
<td>0.73</td>
<td>0.91</td>
<td>0.81</td>
<td>0.81</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>$K_4$</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.023</td>
</tr>
<tr>
<td>$K_5$</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.927</td>
</tr>
</tbody>
</table>

![Fig. 3. Clustering function against water activity for the isolated cuticle of *A. bidwillii* at 25°C. The different values of the cluster function were obtained by Eq. 3 after using the GAB isotherm (Eq. 4). For more details, see text.](image)
cuticle, the lignified material will be localized between the cell wall of the epidermal cells and the innermost region of the cuticle. The innermost cutin layers of cuticles are incrusted with cellulose, hemicellulose and pectins and, for the case of conifer cuticles, in these layers, a synthesis of lignin takes place resulting in a complex and heterogeneous cuticular region.

Lignin material identified in this study (Table 1; Fig. 1) presents the same chemical composition as that found for *P. abies*, i.e. a G-type lignin, mainly formed by units of guaiacyl propane molecules. Since the existence of lignin or lignin-like material has been qualitatively documented in other conifer species (Lendzian et al. 1986), one could ask about the physiological role and other properties of this cuticle fraction. Kögel-Knabner et al. (1994) indicated that the presence of lignin in cuticles of spruce might have a protective function against biodegradation, leading to a selective preservation of cutin during humification in soils and sediments. In this way, Opsahl and Benner (1995) studied a detailed comparison of observed diagenetic trends in lignin and cutin composition of several plant species in relation to the application of these two biopolymers as molecular markers for diagenesis of vascular plant tissues.

The study of the hydrodynamic properties of *A. bidwillii* cuticle can give useful information on the physiological role of the distinct components of this cuticle. Water content sorbed by *A. bidwillii* isolated cuticle is 16.2% of dry weight, the highest in comparison with angiosperm cuticles of the same thickness (approximately 4 μm) such as *Ficus* or *Pyrus* (2.8% and 4.3%), or even thicker, like tomato cuticle (20 μm) (Chamel et al. 1991). However, it is similar to other gymnosperm cuticles like *Abies alba* that sorbs 19.3% of dry weight (Chamel et al. 1992). This clearly indicates that water sorption is not thickness-dependent and that cuticles from gymnosperms have a higher water content than those from angiosperms, regardless of their fruit or leaf cuticles.

Data on water sorption can be discussed in relation to an important physiological property: the cuticular permeability. It has been pointed out that the permeability to water of a biopolymer membrane is proportional to its water concentration in addition to the mobility of water (Chamel et al. 1992, Luque et al. 1995). The amount of water content of *A. bidwillii* cuticle is markedly higher than those previously reported for angiosperm cuticles. It suggests a better permeability to water for conifer cuticles. The most important feature of the present work is that this difference can be assigned to the non-lipidic part of the cuticle, specially its lignin fraction.

The sorption isotherms (Fig. 2) obtained for the cuticle and for the different residues, except for the lignin residue, were very similar to those reported by Chamel et al. (1991) and Luque et al. (1995) for different isolated cuticles. They exhibit a sigmoidal behavior that corresponds to the existence of two sorption phases. In the first one, water molecules form a Langmuir monolayer on highly reactive sorption sites (functional polar groups), this sorption induces pronounced structural rearrangements in the polymer chains allowing more remote binding sites to be accessible. The second one is the formation of a multilayer due to water molecules’ self-association.

The *A. bidwillii* cuticle and the cutin + lignin and hydrolyzable components + lignin residues present, after their estimation with the Hailwood and Horrobin model and, in comparison with other biopolymers, a low number of strong binding sites with a high affinity for water and a relatively important water activity (Table 2). It is interesting to note that the polar residue, probably containing polysaccharides as main component, although it seems to account for an important part of the cuticle strong sites it has a low number of them in comparison with what would be expected by the polysaccharides chemical characteristics. This can be explained because polysaccharide fibers only absorb water in the amorphous regions, most of the hydrogen bonds are engaged in interchain or intramolecular bonds and, therefore, a majority of positions initially available for water absorption are on the oxygen groups (Enderby 1955). Although cutin is almost inactive in water sorption process due to its chemical composition there are a few strong binding sites where water can be sorbed. These sites will be located on the ester and free hydroxyl groups. The presence of lignin in the hydrolyzable components and cutin residues has been ignored in the previous explanations because lignin sorbs water like a perfect solution from very low water activities, indicating that strong binding sites are scarce if present.

On the other hand, the cuticle and the cutin + lignin and polar hydrolyzable components + lignin residues have the same affinity to form water clusters, similar to that found in isolated lignin. Considering that the three former residues also contain lignin it could be concluded that lignin is mainly responsible for water clustering in the cuticle, although the arrangement of the bulk formed by the hydrolyzable components also play an important role. Maximum water content is achieved by the hydrolyzable components + lignin residue higher than the cuticle itself, suggesting that the different components of the cuticle are interlinked and cutin elimination produces more water binding sites. Lignin and cutin + lignin residues have the same water content, indicating that water sorption is very limited in cutin.

Isotherms for each desorption are nearly linear, indicating that water extraction is strictly a partitioning event. These isotherms were always over the sorption ones, which means that the process of water elimination is slower than water sorption, i.e., it is easier to sorb water than to desorb it (Fig. 2). The values of the shrinking factor show that lignin swells during water sorption as well as polysaccharides but, while the latter shrinks easily, the lag in lignin shrinking is higher.

Sorption and desorption isotherms represent relationships between water vapor and the amount of water sorbed by the polymer at equilibrium and constant temperature. When sorption is completely reversible, sorption and desorption isotherms are singular. In practice, sorption is often hysteretic. In order to explain this behavior, it must be considered that some polymer property changes as water levels increase, and that the polymer is both flexible enough to accommodate increasing water, and
sufficiently rigid to sustain irreversible conformational changes. Hysteresis can be then explained by the effect of water on the arrangement of the polymer chains (Giles et al. 1974). Hysteresis can be estimated by the differences in weight, at the same water activity, for sorption and desorption isotherms. The obtained values from data of Fig. 2 showed that the maximum hysteresis took place at 0.6–0.7 of water activity for all the residues. These hysteresis values were higher for lignin (47.2 mg) than for the other residues, 17.9 mg for the cuticle, 19.6 mg for the cutin + lignin, and 25.4 mg for the polar hydrolyzable components + lignin fraction. These values indicate that lignin sorbs water efficiently because it adsorbs an important amount of water easily and retains it during the desorption process. The residue containing hydrolyzable components, on the other hand, have a higher ability for water adsorbing but do not retain it. It is interesting to note that the water activity where hysteresis is higher coincides with the beginning of clustering measured by the Zimm and Lundberg equation (Eq. 3), i.e., at 0.6–0.7 of water activity. This correlation between both events, beginning of water multilayer formation and maximum hysteresis for cuticle, cutin + lignin and hydrolyzable components + lignin residues seems to indicate that water clustering induces a more important rearrangement of the polymer chains. As was pointed out in the Results section, clustering was not measured in the lignin residue since it starts at very low water activities and the precise water activity is very difficult to estimate.

The results obtained in this work agree with the conclusion made by Levine and Slade (1988) in the sense that hysteresis is a characteristic result from a moisture/temperature/time-dependent, slow, non-equilibrium, swelling-related conformational change, which is facilitated by increasing free volume and mobility in a polymer which is being plasticized during sorption that usually progresses through the stage of water clustering.

Taken together, the values of hysteresis, maximum water content and sorption/desorption rate, show the important role played by the lignin residue in water uptake by the cuticle, since it sorbs water efficiently. Although the cuticle fraction composed by polar hydrolyzable components sorbs more water, its capability to retain it is lower than for lignin.

Some aspects of sorption hysteresis are of physiological interest. The cuticle, as the outer barrier of aerial plant organs, may contain a high dose of chemical pesticides or pollutants dissolved in the cuticular water. This liquid phase may act as a sink for these compounds. The capability to retain water may be important in further chemical reactions and diffusion into the plant. On the other hand, the desorptive properties of the plant cuticle may be important with respect to environmental partitioning in that cutinaceous material is resistant to degradation. More specific research is necessary in order to complete our knowledge on this environmental subject.

References


Edited by P. Gardeström