Using Transgenic Poplars to Elucidate the Relationship between the Structure and the Thermal Properties of Lignins

STÉPHANIE BAUMBERGER,*,† PATRICE DOLE,‡ AND CATHERINE LAPIERRE†

UMR de Chimie Biologique, INRA/INA PG, Institut National Agronomique Paris-Grignon, 78850 Thiverval Grignon, France, and UMR Fractionnement des Agro-Ressources et Emballage INRA/URCA, CPCB, Moulin de la Housse, BP 1039, 51687 Reims Cedex 2, France

In an attempt to draw relationships between the molecular structure and the thermal behavior of lignins, thermomechanical analyses were run on six milled wood and enzyme poplar lignin fractions prepared from genetically modified and control woods. All the lignin samples displayed similar thermal profiles with a clear inflection point assigned to the glass transition point. The temperature (T_g) at which this transition occurs showed large variations from 170 to 190 °C, depending both on the genetic modification and on the age of the tree. These variations were found to be closely related to the condensation degree of lignins evaluated by thioacidolysis.

KEYWORDS: Milled wood lignins; thermal analysis; thioacidolysis; transgenic trees

INTRODUCTION

The increasing utilization of lignins and wood products as a source of materials (plastic films, resins, paper, or board) is largely based on thermomechanical molding or pulping processes. The physical state of the lignin polymer during these transformations is crucial because it may strongly affect the quality of the final product, whether it consists of mechanical pulps or synthetic composites. In this context, it is of prime importance to monitor the thermal behavior of lignins, either in situ or after isolation from the plant cell wall.

Like other amorphous polymers, lignins are known to undergo a glass transition corresponding to a softening of the material. The temperature (T_g) at which this transition occurs is influenced by plasticizers such as water (*1*−*3*), by the presence of other polymers such as the polysaccharides of the cell wall (*3*, *4*), and by the structure of the lignin itself. In any case, T_g variations reflect a modification of the molecular mobility resulting from a redistribution of intermolecular bonds and/or from a change in the polymeric skeleton stiffness.

In contrast to the T_g-water content causal relationships, the influence of lignin structure on the thermal properties of isolated lignins has not been extensively investigated. Some studies based on various lignin isolation or fractionation methods have suggested that lignin T_g increases with increasing molecular weight (*5*, *6*), similar to other synthetic or natural polymers (*7*). However, in these studies, T_g variations cannot be assigned to only molecular weight shifts, as many other structural parameters, such as branching and condensation degrees, discriminate the lignin fractions. Other studies on chemically functionalized lignins reported that T_g of isolated lignins is a function of both the frequency and the nature of the substituents (*3*, *8*, *9*).

Genetically modified plants with structurally altered lignins have recently been used to investigate the properties of the lignin matrix within the cell wall (*4*). Although some interesting hypotheses about the cell wall organization were proposed from the thermomechanical behavior of the xylem, such studies could not provide clear-cut relationships between the structure and the macromolecular or thermal properties of lignins.

Our objective was to examine the putative causal relationship between the structure of lignin fractions and their thermal properties, independent of their molecular weight or hydrodynamic volume. We report here on the thermomechanical properties of milled wood and enzyme lignins (MWEL) samples prepared from control trees and from their genetically modified counterparts. The substantial T_g differences displayed by the lignin samples are discussed on the basis of chemical analyses and high-performance size-exclusion chromatography (HPSEC) analyses.

MATERIAL AND METHODS

Lignin Sample Preparation and Chemical Characterization. The wood samples used for lignin isolation and characterization belong to two series referred to as A and B. Series A and series B were isolated from 2-year-old and 6-month-old poplar trees (*Populus deltoides x populus nigra*), respectively. Each series consisted of control samples and their genetically modified counterparts grown in the same conditions. The transgenic trees obtained using antisense constructs are either deficient in CAD (cinnamyl alcohol dehydrogenase) activity (ASCAD samples), in OMT (*O*-methyl transferase) activity (ASOMT), or in both activities (ASCAD × ASOMT). The ASCAD samples of the series A and B differ in the residual CAD activity (respectively, 30% and less than 10% relative to the control level). Milled wood and enzyme lignins were isolated from extract-free wood by a 90/10 (v/v) dioxane/water

* To whom correspondence should be addressed. Tel: (33) 01 30 81 54 64. Fax: (33) 01 30 81 53 73. E-mail: baumberg@grignon.inra.fr.
† UMR de Chimie Biologique, INRA/INA PG, Institut National Agronomique Paris-Gugnon.
‡ UMR Fractionnement des Agro-Ressources et Emballage INRA/URCA, CPCB.

Thermal Properties of Transgenic Poplar Lignins

J. Agric. Food Chem., Vol. 50, No. 8, 2002 **2451**

Table 1. Structural Characteristics and Molecular Weight Distribution (weight-average molecular weight, M_w; number-average molecular weight, M_n; and polydispersity, M_w/M_n) of MWEL Fractions Isolated from 2-Year-Old (series A) and 6-Month-Old (series B) Control and Transgenic Poplar Trees

	control A	ASCAD A	ASOMT A	ASCAD × ASOMT	control B	ASCAD B
isolation yield (%)[a]	37	50	43	59	54	83
β–O–4 (μmol·g^{-1} lignin)[b]	2500	2250	1900	1550	2213	1400
condensation degree (%)[c]	50	55	62	69	56	72
M_w (Da × 10³)[d]	36	26	32	28	28	16
M_n (Da × 10³)[d]	8.7	6.7	7.5	7.2	5.5	4.3
M_w/M_n	4.1	3.8	4.2	3.8	5.6	3.8

[a] Based on the Klason lignin. [b] Units only involved in β-O-4 interunit linkages as determined by thioacidolysis. [c] Molar percentage of total units involved in C–C interunit linkages, calculated from the thioacidolysis yields. [d] Relative values related to polystyrene standards and obtained from HPSEC measurements of acetylated samples.

extraction after ball milling and cellulase hydrolysis (*10*). Thioacidolysis was run according to Lapierre et al. (*11*) with standard error between duplicate experiments in the 3−5% range. The condensation degree (CD) of the lignin preparations, referred to herein as the percentage of lignin units involved in at least one C−C interunit linkage, was estimated from the thioacidolysis yield TY (moles of the main G and S units recovered per gram lignin) as follows:

$$CD = 100 - (TY \times M \times 100)$$

where M is the average molecular weight of lignin unit, taken here equal to 200 g·mol^{-1}, and where TY × M × 100 represents the number of lignin units exclusively involved in β-O-4 linkages per 100 lignin units (*12*).

Thermal Analysis. Thermomechanical analysis (TMA) of lignins was run in the compression mode using a DMA 2980 (TA Instruments) apparatus. Powdered preparations (about 5 mg) were put between two disks and compressed with a constant applied force of 0.1 N, under nitrogen stream. Measurements were carried out between 30 and 200 °C at a heating rate of 3 °C·min^{-1}. T_g values, determined from the TMA curves according to Kubo et al. (*6*), correspond to the temperature at which the powder heap suddenly collapses. The standard error between duplicate T_g determinations is lower than 1%.

HPSEC Analysis. Lignins were acetylated using a 1:2 pyridine/acetic anhydride mixture (v/v, 48 h at room temperature) and recovered according to Gellerstedt (*13*) prior to HPSEC analysis. Solution of acetylated lignins in tetrahydrofuran (about 10 mg·ml^{-1}) were ultrafiltrated on a 0.45-μm PTFE filter (Millipore). HPSEC was performed on a PL-gel column (Polymer laboratories, 5 μm, 600 × 7.5 mm) using nonstabilized THF as eluent at a flow rate of 1 mL·min^{-1}. Detection was made by UV (280 nm), and calibration was based on monodisperse polystyrene standards (Touzart et Matignon) with molecular weight ranging from 800 Da to 1.8 × 10⁶ Da. The number-average molecular weight, M_n, and the weight-average molecular weight, M_w, were calculated by integration of the chromatograms in segments of 0.5 s.

RESULTS AND DISCUSSION

Six different MWEL lignin preparations were obtained with high isolation yields from two control poplar trees and from some of their transgenic counterparts, deficient either in cinnamyl alcohol dehydrogenase (CAD) activity, *O*-methyl transferase (OMT) activity, or in both of these lignin biosynthesis enzymes. These deficiencies which are known to alter the structure of in situ lignin (*14*) similarly affect the characteristics of the isolated lignins. Isolation yields, together with the frequency of β-O-4 linkages and the average molecular weights and polydispersity of the isolated fractions are given in **Table 1**.

In an attempt to evaluate the impact of these structural variations on the glass transition T_g, the lignin samples were submitted to thermomechanical analysis. All the TMA curves obtained in the compression mode show the same global profile, with a T_g ranging from 170 °C to 190 °C, depending on the genetic modification and the cultivation experiment (**Figure 1**). Although less pronounced than those of lignins recovered after
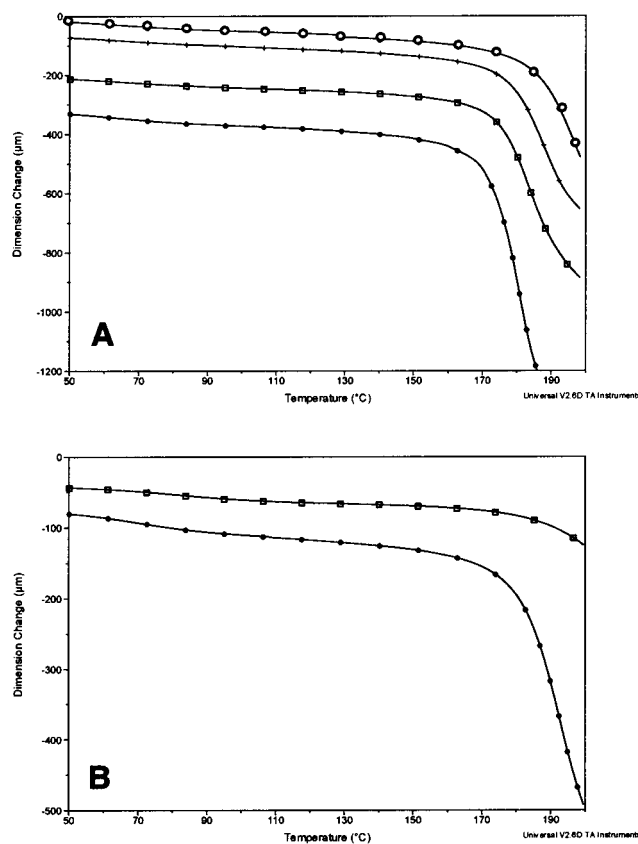

Figure 1. Thermomechanical analysis of poplar MWEL samples prepared from control trees and their transgenic counterparts. Series A: control A (●), ASCAD A (□), ASOMT A (+), ASCAD × ASOMT (○). Series B: control B (●), ASCAD B (□). The curves show the collapse of the compressed (0.1 N) powder heap sample submitted to a 3 °C min^{-1} heating.

severe chemical alterations (*8*), these T_g variations are substantial when compared to the standard error between two T_g determination (which was found to be lower than ±1 °C). The values of 171 and 181 °C relative, respectively, to control A and control B are in agreement with the 179 °C T_g previously measured by DSC on a poplar MWEL lignin sample (*15*). Lower T_g values ranging between 60 and 90 °C have been previously obtained by dynamic thermal analysis (*2*). These values are not inconsistent with our results because they were measured in water-saturated conditions. The 10 °C difference observed between control A and control B is not unexpected, as these lignin samples were obtained from trees grown in different conditions and collected at different ages. This result points to the importance of comparing samples belonging to the same series. Such a comparison reveals that all the genetic modifications
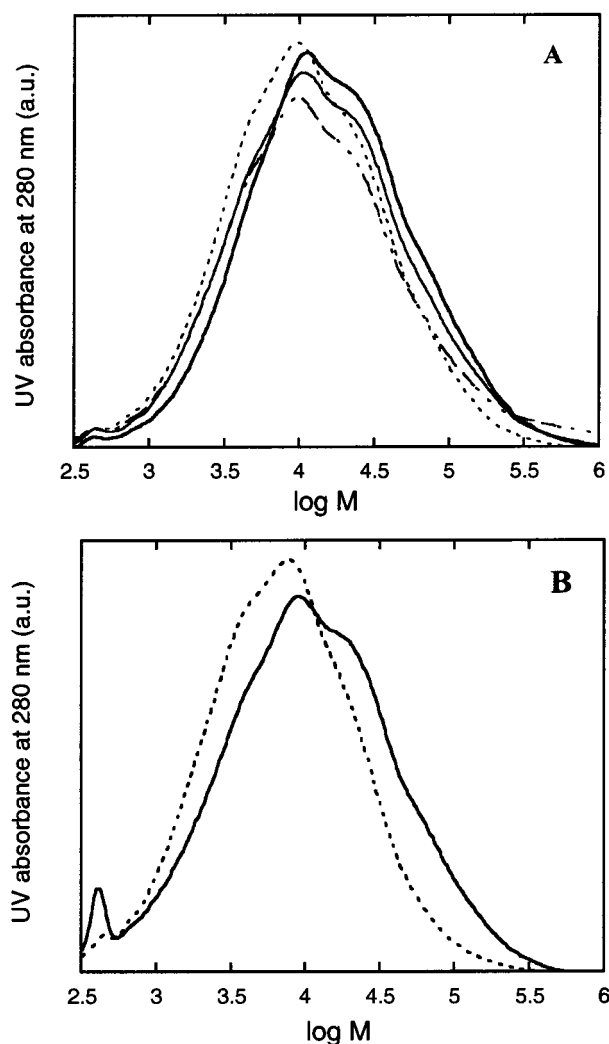
2452 *J. Agric. Food Chem.,* Vol. 50, No. 8, 2002

Baumberger et al.

Figure 2. High-performance size-exclusion chromatography of acetylated lignin samples isolated from control (thick—), CAD-deficient (- - -), OMT-deficient (–), and both CAD- and OMT- deficient (-–) poplar trees belonging to two 2-year-old (A) and 6-month-old lines (B). Calibration is made using polystyrene standards and all the curves are normalized with respect to the total area under the curve.

investigated here lead to MWEL with a higher T_g than that of the control samples.

From a general point of view, the parameters most likely to affect the T_g values of polymers are the presence of contaminants (such as water and sugars), the molecular weight, and the chemical structure. The lignin samples prepared and stored in the same conditions do not differ by their water content. As a consequence of the enzymatic isolation step, the lignin contamination by carbohydrates was found very low and in the same proportion in all the samples (^{13}C NMR signals of anomeric C-1 hardly discernible from the baseline (*16*)). Intrinsic structural features of the lignin polymer, thus, are very likely to account for the observed T_g variations.

Comparison of the different HPSEC absorbance-log M curves (**Figure 2**) indicates similar molecular weight profiles. In agreement with literature data on MWEL lignin preparations isolated in similar conditions (*10*, *17*, *18*), all the lignin samples investigated herein displayed relatively high average molecular weights (M_w ranging between 16.0 and 36.0 × 10^3 Da) and a low polydispersity (4.2 ± 0.7) (**Table 1**). The lower molecular weight of the lignins of the B series most probably reflects the earlier lignification stage of the B lines. Lignins isolated from
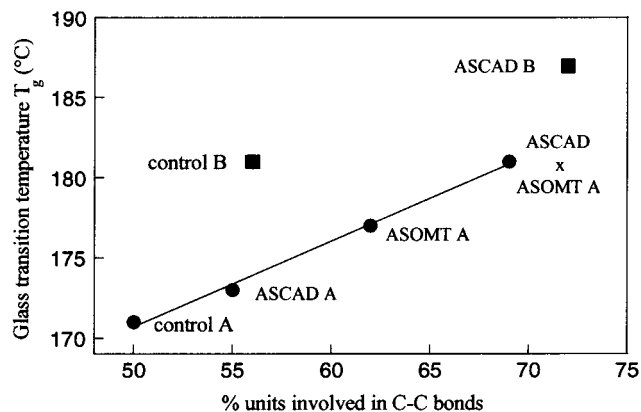

Figure 3. Correlation between the thermal properties (T_g) and the condensation degree (% C_6C_9 units involved in C–C linkages) of milled wood and enzyme lignins isolated from control and transgenic poplars. Series A and B correspond to two different cultivation experiments run for distinct transgenic and control lines.

CAD- or OMT-deficient trees systematically displayed lower M_w values than that of the control sample (11–29% relative reduction). This tendency is more pronounced in the case of the CAD deficient samples ASCAD A, ASCAD × ASOMT, and ASCAD B. The variation of polydispersity does not exceed 17% and is apparently not correlated with the genetic modification.

In contrast to nonlignin components and to lignin molecular weight distribution, the lignin condensation degree (evaluated herein as the percentage of lignin units involved in a condensed bonding pattern) allowed us to very distinctly discriminate the lignin samples. Indeed, whereas the percentage of lignin units involved in C–C bonds is only 50% in the control sample of the A series, it rises up to 69% in the sample isolated from the most severely altered ASCAD × ASOMT line, with intermediate values observed for the single ASCAD and ASOMT samples (**Figure 3**). C–C interunit linkages are supposed to reinforce the polymer skeleton, thereby reducing its molecular mobility. In agreement with this hypothesis, **Figure 3** shows a strikingly clear correlation between the condensation degree and the T_g of the lignin sample belonging to the same series. Although the possible contribution of functional groups cannot be ruled out, the condensation degree appears thus to primarily influence lignin T_g. In the case of synthetic polymers, as well as in the case of starch polysaccharides, a linear relationship between M_n and T_g has been established (*7*). In these cases, the observed T_g increase with increasing M_n is assigned to the decrease of free volume (*19*) and reaches a plateau at a molecular weight corresponding to the polymer entanglement limit (limit M_n ranging between 1250 and 19000 for many pure synthetic amorphous linear polymers). The lack of correlation between the T_g of the lignin samples investigated in our study and their molecular weight distribution most probably results from the high values of the average molecular weights.

CONCLUSION

This study reveals that milled wood and enzyme lignin samples showing similar molecular weight distribution and high average molecular weights can be advantageously used to investigate the influence of lignin structure on their physical properties. Increase in the glass transition temperature of lignin isolated from genetically modified poplar trees can be clearly correlated with the condensation degree of lignins. When associated with thioacidolysis, thermomechanical analysis proves

Thermal Properties of Transgenic Poplar Lignins

J. Agric. Food Chem., Vol. 50, No. 8, 2002 **2453**

to be a sensitive method particularly efficient at deciphering lignin structure−property relationships.

LITERATURE CITED

(1) Back, E. L.; Salmen, N. L. Glass transitions of wood components hold implications for molding and pulping processes. *Tappi J.* **1982**, *65*, 107−110.

(2) Irvine, G. M. The glass transition of lignin and hemicellulose and their measurement by differential thermal analysis. *Tappi J.* **1984**, *67*, 118−121.

(3) Hatakeyama, H. Thermal analysis. In *Methods in Lignin Chemistry*; Lin, Y., Dence, C. W.; Eds.; Springer-Verlag: Berlin/ Heidelberg, 1992; pp 200−214.

(4) Hepworth, D. G.; Vincent, J. F. V.; Schuch, W. Using viscoelastic properties of the woody tissue from tobacco plants to comment on the molecular structure of cell walls. *Ann. Bot.* **1998**, *81*, 729− 734.

(5) Yoshida, H.; Mörck, R.; Kringstad, K. P.; Hatakeyama, H. Fractionation of kraft lignin by successive extraction with organic solvents. *Holzforschung* **1987**, *41*, 171−176.

(6) Kubo, S.; Uraki, Y.; Sano, Y. Thermomechanical analysis of isolated lignins. *Holzforschung* **1996**, *50*, 144−150.

(7) Slade, L.; Levine, H. Water and the glass transition. Dependence of the glass transition on composition and chemical structure: special implications for flour functionality in cookie baking. *Crit. Rev. Food Sci. Nutr.* **1991**, *30*, 115−360.

(8) Kelley, S. S.; Glasser, W. G.; Ward, T. C. Characterization of chain extended hydroxypropyl lignins. *J. Wood Chem. Technol.* **1988**, *8*, 341−359.

(9) Rials, T. G.; Glasser, W. G. Phase morphology of hydroxypropylcellulose blends with lignin. In *Lignin Properties and Materials*; Glasser, W. G., Sarkanen, S., Eds; American Chemical Society: Washington, DC, 1989; pp 464−475.

(10) Chang, H. M.; Cowling, E. B.; Brown, W.; Adler, E.; Miksche, G. Comparative studies on cellulolytic enzyme lignin and milled wood lignin of sweetgum and spruce. *Holzforschung* **1975**, *29*, 153−159.

(11) Lapierre, C.; Pollet, B.; Petit-Conil, M.; Toval, G.; Romero, J.; Pilate, G.; Leplé, J−C.; Boerjan, W.; Ferret, V.; De Nadai, V.; Jouanin, L. Structural alterations in transgenic poplars with depressed cinnamyl alcohol dehydrogenase or caffeic acid-*O*-methyltransferase activity have an opposite impact on the efficiency of industrial kraft pulping. *Plant Physiol.* **1999**, *119*, 153−163.

(12) Lapierre, C.; Pollet, B.; MacKay, J. J.; Sederoff, R. R. Lignin structure in a mutant pine deficient in cinnamyl alcohol dehydrogenase. *J. Agric. Food Chem.* **2000**, *48*, 2326−2331.

(13) Gellerstedt, G. Gel permeation chromatography. In *Methods in Lignin Chemistry*; Lin, Y., Dence, C. W.; Eds.; Springer-Verlag: Berlin/Heidelberg, 1992; pp 487−497.

(14) Boudet, A. M.; Lapierre, C.; Grima-Pettenati, J. Transley Review N*o*. 80. Biochemistry and molecular biology of lignification. *New Phytol.* **1995**, *129*, 203−236.

(15) Lapierre, C.; Monties, B.; Gonthier, A. V.; Dworkin, A. Differential calorimetric study of pine and poplar lignins between 300 and 525 K. *J. Appl. Polym. Sci.* **1986**, *32*, 4561−4572.

(16) Robert, D.; Piersantelli, D.; Jouanin, L.; Ferret, V.; Pollet, B.; Lapierre, C. ^{13}C NMR spectroscopy of lignins from transgenic poplars deficient in *O*-methyltransferase and cinnamyl alcohol dehydrogenase. In *Proceedings of the 10th ISWPC, June 7−10, 1999, Yokohama, Japan*; Vol III, pp 18−21.

(17) Koch, H.; Hübner, K.; Fisher, K. The influence of light on the molecular mass of lignins. *J. Wood Chem. Technol.* **1994**, *14*, 339−349.

(18) Ben-Ghedalia, D.; Yossef, E. Effect of isolation procedure on molecular weight distribution of wheat straw lignins. *J. Agric. Food Chem.* **1994**, *42*, 649−652.

(19) Ferry, J. D. *Viscoelastic Properties of Polymers*, 3rd ed.; John Wiley & Sons: New York, 1980.