Nuclear Magnetic Resonance Relaxation Studies of Plant Polyester Dynamics. 2. Suberized Potato Cell Wall

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ABSTRACT: Results of magic-angle-spinning (MAS) ^{13}C NMR experiments are reported for the biopolyester suberin grown within potato cell walls. Measurements of the proton rotating-frame relaxation time, $T_{1\rho}(H)$, indicate that wound healing involves the formation of spatially separated suberin and cell-wall domains. Suberin appears to be attached to the cell-wall domain at discrete sites, with both its methylene and phenylpropanoid groups divided into two populations having distinct chemical shifts and spin-relaxation characteristics. Measurements of $T_{1\rho}(C)$ and $T_1(C)$ in this biopolymer system show significant site-specific and tissue-specific variations. Taken together, these relaxation experiments reveal a shift of spectral density for the cell wall, upon suberization, toward mid-kilohertz motions which may enhance cuticular resiliency.

Introduction

Suberin is a polymer that grows within plant cell walls as a wound-healing response and is thought to prevent invasion of the tissue by fungal and bacterial pathogens.[1,2] Although it is known that the polyester is deposited on the wound surface within several days, no direct information is available describing the covalent linkages through which suberin attaches to or becomes embedded within the carbohydrate cell-wall matrix. Neither is it established which molecular events occurring during suberization might change the bulk mechanical properties of the plant cell wall, altering in turn its effectiveness as a protective barrier.

A number of recent studies have used solid-state ^{13}C nuclear magnetic resonance (NMR) to characterize plant polymers such as lignin, cell-wall carbohydrates, and the cuticular materials cutin and suberin.[3-8] Such NMR studies have been able to directly identify and quantitate the chemical functionalities of these polymers in intact plant materials. Drawing on the essential link between polymer dynamics and bulk mechanical properties,[9] NMR spin-relaxation measurements have helped to generate an informative dynamic profile for lime cuticle and its two polymeric constituents, cutin and wax.[10]

In the present study we report related magic-angle-spinning ^{13}C NMR studies of the molecular dynamics of suberized cell wall from wounded potato tissue. Results are presented for a series of $T_{1\rho}(H)$, $T_{1\rho}(C)$, and $T_1(C)$ relaxation experiments which yield information on polyester–carbohydrate molecular organization as well as dynamic behavior at both mid-kilohertz and megahertz frequencies. Both the motional behavior and the mixing behavior that characterize the assembly of suberin–carbohydrate complexes in wounded potato contrast sharply with the organization of cutin–wax complexes in lime cuticle. The spin-relaxation data for this biopolymer system support the proposal that suberin deposition involves formation of chemical bonds to the cell wall.

Materials and Methods

Potato Tissue. Using published procedures that included sterile precautions,[8,11] potato tubers (*Solanum tuberosum L.* cv. Russet Burbank) were peeled, cut into 1 × 1 × 4 cm sections, and aerated for 7 days at 20 °C to suberize the wound-healing tissue. To remove unsuberized cellulose and pectin the material was treated with cellulase and pectinase (Sigma Chemical Co., St. Louis, MO). At least 90% of the unsuberized polysaccharides were removed by these treatments. Finally, soluble lipids were removed by extraction with a 2:1 (v/v) mixture of methylene chloride and methanol. Typically, 4.5 g of suberized potato cell wall was obtained from 22 kg of potatoes. The soluble products of chemical depolymerization were analyzed by ^{13}C NMR,[8] confirming the similarity of our materials to the potato suberin described earlier by Kolattukudy et al.[11] By omitting the 7-day incubation period from the above protocol, a sample of residual unsuberized cell wall was obtained.

NMR Spectra. Solid-state ^{13}C NMR experiments were performed at ambient temperature (26 °C) on two spectrometers: an IBM Instruments WP-200 (^{13}C resonance frequency of 50.33 MHz) equipped with high-power amplifiers and a probe from Doty Scientific (Columbia, SC) and a home-built instrument (^{13}C resonance frequency of 31.94 MHz). Potato samples were dried, ground with a Wig-L-Bug amalgamator (Spex Industries), and packed into cylindrical, double-bearing rotors. Cross-polarization magic-angle-spinning (CPMAS) ^{13}C NMR spectra were obtained at 31.94 MHz using 2-ms, 50-kHz ^{13}C–^{1}H spin-lock contacts, high-power (65-kHz) proton decoupling, and magic-angle spinning at a speed of 3 kHz. Spectra at 50.33 MHz were collected with spin-lock/decoupling fields of 48 kHz and a spinning speed of 5 kHz. A recycle delay of 1 s was inserted between successive data acquisitions. For rotating-frame relaxation measurements, contact times and rf field strengths were varied as described below.

Direct-polarization magic-angle-spinning (DPMAS) ^{13}C NMR spectra were acquired at 31.94 MHz following a single ^{13}C 90° pulse. A frequency-modulated, continuous-wave ^{1}H decoupling field of 5 kHz was used, and the recycle delay between acquisitions was 2.5 s, >5 times the spin–lattice relaxation time for methylene carbons.

Spin-Relaxation Measurements. Proton rotating-frame relaxation times, $T_{1\rho}(H)$, were determined from the decay of the carbon signal as a function of ^{13}C–^{1}H contact time (τ) in a CP-MAS experiment.[6,12] The $\langle T_{1\rho}(H)\rangle$ values were calculated from least-squares fits of log (carbon signal height) vs τ, using values of τ = 1.5–8 ms, unless otherwise noted. Results of these variable contact-time experiments were also used to derive suberin's functional-group composition. Carbon spin–lattice relaxation times, $T_1(C)$, were measured by monitoring the recovery of ^{13}C magnetization following cross polarization and inversion.[13] Values of $\langle T_1(C)\rangle$ were determined from the initial rates of this recovery. Carbon rotating-frame relaxation times, $T_{1\rho}(C)$, were measured by recording the ^{13}C signal as a function of carbon spin-lock time following cross polarization. The spin-lock field

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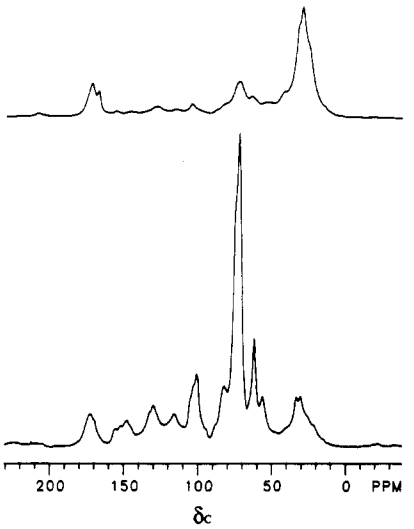


Figure 1. CPMAS ^{13}C NMR spectra at 31.94 MHz of polyesters from intact plant tissue: (bottom) wound periderm from Russet Burbank potatoes, 297 mg, 75 000 transients; (top) cutin from limes, 286 mg, 50 000 transients. Both spectra were collected using 2-ms, 50-kHz ^{13}C–^{1}H spin-lock contacts with high-power proton decoupling ($\gamma B_2(H)/2\pi$ = 65 kHz), magic-angle spinning at 3.0 kHz, and a 1-s recycle delay between transients. The spectra were processed with a digital line broadening of 40 Hz and plotted with vertical-scale adjustment to equalize the heights of the carboxyl peaks at 172 ppm. Both samples were treated similarly to remove cellulose, pectin, and waxes. More extensive purification protocols result in some attenuation of the cutin CHO resonance at 72 ppm and the potato cell-wall resonances between 62 and 101 ppm.[16]

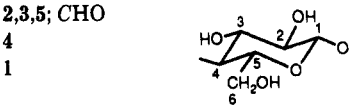
$B_1(C)$ was varied between 37 and 60 kHz in separate relaxation experiments. Values of $\langle T_{1\rho}(C)\rangle$ were determined from a least-squares fit of log (carbon signal height) vs spin-lock time over the first 1.0 ms of signal decay.[14,15]

Results and Discussion

A. Carbon Types. As reported previously[6-8] and illustrated in Figure 1, the CPMAS ^{13}C NMR spectra of both cutin from limes and suberin from potatoes exhibit signals typical of aliphatic–aromatic polyesters: bulk methylenes, oxygenated methylenes and methines, aromatics or olefinics, and carboxyl groups. Since suberin is inseparable from the cell wall, the spectrum of potato wound periderm (Figure 1, bottom) also contains prominent carbohydrate resonances, some of which overlap signals from the suberin. The carbohydrate signals in this spectrum are relatively narrow, though the peaks from the cell-wall mixture of cellulose, pectin, and hemicellulose are not as well resolved as those of the corresponding pure materials.[17,18] Signals are observed from two types of suberin methylenes (δ_C 30 and 33 ppm), reflecting differences in either the polyester chain lengths or the distances of these chains from points of covalent attachment to the cell wall. The published spectral assignments for suberized potato tissue[7,8] are repeated in Table I to facilitate the subsequent discussion.

Although cutin and suberin are both plant-cuticle polyesters, they differ substantially in terms of chemical composition. Comparisons of CPMAS and DPMAS spectra, the former adjusted for cross-polarization efficiency and the latter for nuclear Overhauser enhancements, allow us to account quantitatively for all carbon atoms.[6] The liquidlike methylenes, observable with low-power decoupling, amount to 59% in lime cutin but only 14% in lime cuticle and suberized potato. Approximately half of the potato sample consists of cell wall; the remaining suberin in the tissue is composed of aromatics and alkenes

Table I
^{13}C NMR Assignments for Suberized Potato Tissue

carbon type[a]	shielding,[b] ppm
$(CH_2)_n$	30,[c] 33
OCH_3	56[c]
6	62
2,3,5; CHO	72
4	83
1	101[d]
	116, 130,[c] 150[c]
COO	172[c]

[a] Assignments based on ^{13}C NMR spectra of authentic sugars, carbohydrates, and polyesters,[3,5,17-19] spectra of unsuberized potato,[8] and delayed-decoupling CPMAS identification of nonprotonated and mobile carbons.[20-22] Polyester carbons are italicized; carbohydrate carbons are in boldface. [b] Values obtained from Figure 1 and referenced externally to tetramethylsilane. [c] Retained in the spectrum if a 50-μs delay is inserted prior to ^{1}H decoupling. [d] Includes minor contribution from unsaturated suberin carbons.

that together outnumber the bulk methylene groups 2:1.[8] The suberin compositional profile contrasts sharply with that for lime cutin: the latter polymer has a corresponding unsaturated-to-methylene ratio of 1:29. Thus ^{13}C NMR of the intact plant materials confirms prior analyses of their partial degradation products, which indicated that lignin, suberin, and cutin span a range from highly aromatic to highly aliphatic polyesters.[1,6-8,23,24]

B. Polymeric Domains. The proton spin-relaxation parameter $T_{1\rho}(H)$, which is sensitive to mid-kilohertz motions, can provide information about cooperative overall undulations of the suberized cell wall. $T_{1\rho}(H)$ experiments may also be used to probe domain formation within the cell wall in a manner that is well established for the study of phase separation in polymer blends.[14,25] Spin diffusion among protons which are proximate in space causes them to behave as a single spin reservoir, characterized by a single, averaged $\langle T_{1\rho}(H)\rangle$. The observation of distinct $\langle T_{1\rho}(H)\rangle$ values, measured from the contact-time dependence of signal intensities of different carbons, indicates that the protons associated with these carbons belong to distinct spin reservoirs which are separated in space. The 10-ms time scale of the spin-lock period makes $T_{1\rho}(H)$ sensitive to domains which are 50 Å or larger in size.[26]

Figure 2 shows representative plots of carbon signal height vs ^{13}C–^{1}H contact time for the cell-wall and suberin components of potato wound periderm. $T_{1\rho}(H)$ results for this sample and several reference materials are summarized in Table II. Values of $\langle T_{1\rho}(H)\rangle$ for both cutin in cuticle and suberin in wound periderm, while comparable to values for various woody plants,[3] are shorter than those reported for lignin and many synthetic glassy polymers.[14,24] Thus mid-kilohertz motions are more important for these biopolyesters than for many materials of similar chemical structure. The observation of consistently longer $T_{1\rho}(H)$'s for suberin than cutin suggests that the cell-wall matrix of potato tissue imparts more dynamic restrictions on this time scale than does the wax in assembled cuticle. Cell-wall values for both suberized and unsuberized potato samples are intermediate between the $T_{1\rho}(H)$'s measured for amorphous plant starches[5,17] and for the crystalline portions of cellulose acetate.[27] A shortening of $\langle T_{1\rho}(H)\rangle$ for the cell-wall component accompanies suberin growth, reflecting a significant enhancement of its mid-kilohertz motions.

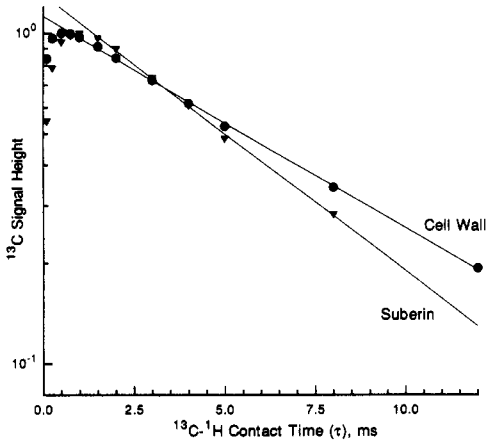


Figure 2. Semilogarithmic plot of ^{13}C NMR signal heights vs CP contact time, τ, for polyester and carbohydrate constituents of potato wound periderm. Suberin unsaturated groups at 130 ppm (●); cell-wall CH_2O's at 62 ppm (▼). The straight lines are least-squares fits of the signal decay over the range τ = 1.5–8 ms, whose slopes yield values of $\langle T_{1\rho}(H)\rangle$.

Table II
^{1}H Rotating-Frame Relaxation Parameters for Carbons in Potato Tissue

		$\langle T_{1\rho}(H)\rangle$,[a] ms		
chem shift, ppm	carbon type[b]	cutin–wax	suberized potato	unsuberized potato
30	$(CH_2)_n$	3.8	5.0	
33	$(CH_2)_n$	3.9	4.5	
62[c]	**6**	3.8	**6.6**	**9.3**
72[c]	**2,3,5**; *CHO*	4.0	**6.6**	**8.2**
101[d]	**1**		**6.6**	**9.1**
116	unsaturated		5.3	
130	unsaturated		5.2	
172[c]	*COO*	5.4	4.9	

[a] From straight-line fits of log (^{13}C signal height) vs cross-polarization contact time, τ. For all potato-tissue carbons, $\langle T_{1\rho}(H)\rangle$ is determined from the decay of the ^{13}C signal using τ = 1.5–8 ms. The cutin–wax data, which have been reported previously,[10] are fits over similar time intervals. These results, obtained at 31.94 MHz, have an estimated uncertainty of 15%. [b] Polyester carbons are italicized; carbohydrate carbons are in boldface and refer to the structure shown in Table I. [c] In lime cuticle, resonances from oxygenated carbons are found at 64, 73, and 174 ppm. [d] Includes minor contribution from unsaturated suberin carbons.

How are the two chemical constituents mixed together in suberized potato cell wall? Suberin protons belong to one spin reservoir and possess a common $\langle T_{1\rho}(H)\rangle$ of 5.0 ms as measured through several different suberin carbon resonances. Carbohydrate protons belong to a second spin reservoir having a $\langle T_{1\rho}(H)\rangle$ of 6.6 ms. In neither material does molecular motion appear to attenuate ^{1}H–^{1}H interactions significantly. Despite the fact that spin diffusion is efficient *within* each polymeric constituent of the potato periderm, their distinct values of $T_{1\rho}(H)$ indicate the poor communication *between* suberin and cell-wall domains.[7] By contrast, lime cuticle exhibits incomplete spin diffusion among protons of the cutin component but a common $\langle T_{1\rho}(H)\rangle$ for both cutin and wax methylenes,[10] and pine wood is characterized by a single $\langle T_{1\rho}(H)\rangle$, suggesting intimate mixing of its lignin and cellulose components.[3] Structural hypotheses based on the suberin spin-relaxation results are developed further in the following discussion.

C. Mid-Kilohertz Motions. Like the proton spin-relaxation parameter $T_{1\rho}(H)$, the carbon rotating-frame relaxation time, $T_{1\rho}(C)$, reflects motions occurring at mid-kilohertz frequencies in amorphous polymers.[28-30] Unlike $T_{1\rho}(H)$, however, $T_{1\rho}(C)$ can provide site-specific motional information because individual $\langle T_{1\rho}(C)\rangle$ values are not averaged by spin diffusion. As noted previously,[8,9] the slow overall motions probed by $T_{1\rho}(C)$ may be related to the resiliency or brittleness of cuticular materials. In heterogeneous biopolymers such as suberized cell wall, a distribution of motional rates is typically observed for each carbon type, resulting in nonexponential plots of ^{13}C signal intensity vs spin-lock time in the $T_{1\rho}(C)$ experiment.[8,28]

Table III compares the average parameters $\langle T_{1\rho}(C)\rangle$ for cuticle and suberized cell wall, revealing a number of site-specific and tissue-specific trends. For both plant samples, the efficiency of mid-kilohertz motions is greatest for the $(CH_2)_n$ groups of long acyl chains, intermediate for CH_2O moieties, and least for CHO carbons. For both oxygenated functional groups (62 and 72 ppm), the $\langle T_{1\rho}(C)\rangle$ values depend additionally on whether the moieties are contained within the carbohydrate or polyester material (compare adjacent entries at each spin-lock field). Thus mid-kilohertz motions are much less facile for carbons of the sugar ring in suberized potato cell wall than for either CH_2O or CHO carbons in the lime cuticle. If the cell wall is unsuberized, both $\langle T_{1\rho}(H)\rangle$ and $\langle T_{1\rho}(C)\rangle$ indicate that mid-kilohertz motions of the sugar groups are less efficient still.

A detailed comparison of the $T_{1\rho}(C)$ behavior for chain methylenes of related polymers is displayed in Figure 3. The two suberin $(CH_2)_n$ resonances show similar $\langle T_{1\rho}(C)\rangle$ values at each spin-lock field, despite their contrasting dynamic behavior at megahertz frequencies (vide infra). Both groups of suberin methylenes exhibit rotating-frame relaxation times and B_1 dependencies that closely match the wax constituent of lime cuticle.[10] The mid-kilohertz motions evidenced for bulk methylene groups in suberin are somewhat less facile than in cutin (either alone or within cuticle) but much more efficient than in a synthetic polyester such as poly(butylene terephthalate).[10,29] As compared with cutin, the partial restriction of overall motions inferred from the present results suggests a structural arrangement in which aliphatic chains of the suberized potato tissue are associated with and/or bound to the cell-wall matrix.

D. Megahertz Motions. Local segmental motions at megahertz frequencies, which are thought to determine the bulk modulus and other mechanical properties of solid polymers,[9,15] may be monitored conveniently through the carbon spin–lattice relaxation time, $T_1(C)$. If both liquidlike and solidlike groupings are present in the material (vide supra), their respective relaxation times may be measured separately. As demonstrated for lime cutin and cuticle,[6-8] the dynamic information obtained from $T_1(C)$ experiments may also permit structural inferences to be drawn regarding the presence of polyester cross-links and cutin–wax interactions.

Average spin–lattice relaxation times at two magnetic field strengths are summarized in Table IV for the solidlike carbons of suberized potato and related plant materials. As expected from prior spin-relaxation studies of cutin and cuticle, the bulk methylene groups exhibit greater segmental mobility than do unsaturated or carbonyl carbons of the suberin polyester. Aliphatic carbons bound to oxygen, and particularly those within sugar rings of the cell-wall component, generally show greater motional restrictions at megahertz frequencies. Aromatic and doubly bonded carbons display intermediate dynamic behavior as judged from values of $\langle T_1(C)\rangle$ (but see below). These site-specific trends in segmental mobility parallel the overall (mid-kilohertz) dynamics observed for various functional groups from $\langle T_{1\rho}(C)\rangle$.

Table III
^{13}C Rotating-Frame Relaxation Parameters for Carbons in Potato Tissue

		$\langle T_{1\rho}(C)\rangle$,[a] ms							
		37 kHz		44 kHz		50 kHz		60 kHz	
chem shift, ppm	carbon type[b]	C[c]	S[c]	C	S	C	S	C	S
30	$(CH_2)_n$	2.3	2.7	2.6	3.1	2.8	3.8	3.3	4.4
33	$(CH_2)_n$	2.5	2.9	3.2	3.5	3.7	4.2	4.5	5.0
56	OCH_3		7.6				13.4		12.3
62[d]	**6**	2.8	**3.3**	3.4	**6.3**	3.6	**7.8**[e]	4.5	**9.8**
72[d]	**2,3,5**; CHO	5.3	**9.9**	7.9	**17.7**	7.5	**21.4**[e]	9.9	**28.0**
101	1		**11.9**				**19.1**		**25.9**
116	unsaturated		6.4		6.5		6.2		7.7
130	unsaturated		7.2		8.3		8.8		9.4

[a] From short-time behavior (0.05–1.00 ms) of the ^{13}C magnetization held in the indicated rf fields after spin locking and cross polarization from ^1H. These values, measured at a ^{13}C resonance frequency of 31.94 MHz, have an estimated uncertainty of 15%. [b] Polyester carbons are italicized; carbohydrate carbons are in boldface and refer to the structure shown in Table I. [c] C refers to lime cuticle;[10] S refers to suberized potato. Some of the potato results have been reported previously.[7] [d] In lime cuticle, resonances from oxygenated carbons are found at 64 and 73 ppm. [e] Values for unsuberized potato are 9.5 and 24.5 ms for peaks at 62 and 72 ppm, respectively.

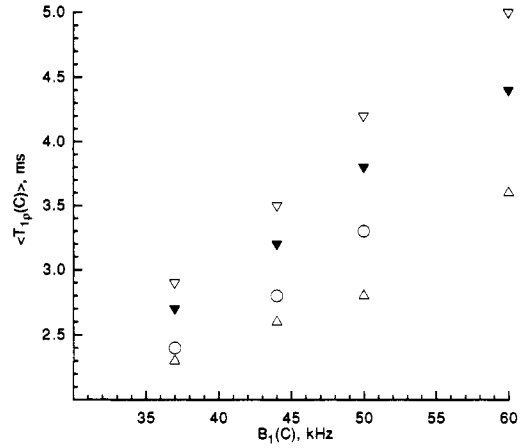


Figure 3. Average carbon rotating-frame relaxation times, $\langle T_{1\rho}(C)\rangle$, as a function of spin-lock field, $B_1(C)$, for methylene carbons in plant-polyester materials. Cutin: alone (○) and intact cuticle (△); suberin: 30-ppm signal (▽) and 33-ppm signal (▼).

The 50-MHz measurements for $(CH_2)_n$'s at 30 ppm yield similar short values of $\langle T_1(C)\rangle$ for cutin, cuticle, and suberin.[10] Since nearly 90% of both the cuticle and suberin NMR signals may be classified as solidlike, the spin–lattice relaxation parameters derived from CP experiments may be taken to indicate a similar degree of segmental mobility for their 30-ppm methylene groups. Nevertheless, $\langle T_1(C)\rangle$ for the suberin resonance at 33 ppm exceeds the values found for both wax methylenes in lime cuticle and 30-ppm methylenes in the identical suberized potato. The population of motionally restricted suberin methylenes appearing at 33 ppm also experiences strong enough ^{13}C–^1H dipolar interactions to completely dephase its ^{13}C NMR signals in delayed-decoupling CPMAS experiments. Evidence from both $\langle T_1(C)\rangle$ and delayed-decoupling measurements is then consistent with the suggestion[1,7,8] that some portions of the polymer chain are closer than others to discrete anchoring sites between suberin and the plant cell-wall carbohydrates. Those aliphatic residues more distant from cell-wall anchors may be embedded within the wax component of intact wound-healing tissue.

The motional restriction of carbohydrate segments is quite evident in the long values of $\langle T_1(C)\rangle$ found for the CHO resonance at 72 ppm. Spin–lattice relaxation of analogous groups in related biopolymer systems indicates, for instance, less segmental motion for sugar rings in noncrystalline cellulose,[30,31] a similar degree of constraint for polyester cross-links in lime cutin,[6] and more facile segmental motion in lime cuticle.[10] In the absence of refined spectral assignments for these heterogeneous materials, more detailed motional comparisons are not possible. Nonetheless, our determinations of $\langle T_1(C)\rangle$ for both potato samples at 31.94 MHz show that suberization imposes additional restrictions on the cell-wall segmental motions, as expected if wound healing involves growth of a polyester within the carbohydrate matrix. Exactly the opposite trend is observed for intact lime cuticle, where the presence of wax dramatically enhances local motions of cutin cross-links.

Finally, the aromatic and olefinic groups of suberin display complex, nonexponential spin–lattice relaxation behavior (Figure 4), indicating that the average $\langle T_1(C)\rangle$ includes contributions from a sizeable population of highly immobilized groups. Thus some fraction of the phenylpropanoid moieties, perhaps that which is anchored by covalent bonds to cell-wall carbohydrates, becomes motionally restricted. The observation of mobile and restricted segments for a given carbon type is similar to that noted above for $(CH_2)_n$ groups, though the broad phenylpropanoid resonances are not separated cleanly according to chemical shift.

Conclusions

These studies demonstrate the power of CPMAS ^{13}C NMR in general, and spin-relaxation methods in particular, for providing detailed structural and dynamic information about complex mixtures of plant biopolymers. The results obtained for suberized potato cell wall differ importantly from those obtained for functionally related lime cuticle as regards both molecular organization of the polymeric components and the motional impact which these components have upon one another within intact plant assemblies.

The formation of spatially separated suberin and cell-wall domains in wound periderm tissue has been deduced from measurements of $T_{1\rho}(H)$, which indicate efficient ^1H–^1H crosstalk *within* each molecular constituent but poor spin communication *between* the two types of polymeric species. These results contrast sharply with those for lime cuticle. In the cuticle, spin diffusion was found to be incomplete for the protons of cutin while similar values of $\langle T_{1\rho}(H)\rangle$, $\langle T_{1\rho}(C)\rangle$, and $\langle T_1(C)\rangle$ for the aliphatic chains of cutin and wax suggested that the hydrophobic groupings were closely associated.[10]

If there exist suberin-rich and carbohydrate-rich patches within the wound-healing potato tissue, then it is reasonable that the electronic environment and molecular motion of a given chemical moiety could be modulated by its distance from a polymer–polymer interface. Such behavior is observed for two types of suberin function-

Table IV
^{13}C Spin-Lattice Relaxation Parameters for Carbons in Suberized Potato Tissue

chem shift, ppm	carbon type[b]	⟨T_1(C)⟩,[a] ms: cutin 50 MHz[c]	cutin-wax 50 MHz[c]	suberized potato 50 MHz	suberized potato 32 MHz	unsuberized potato 32 MHz
30	$(CH_2)_n$	190	120	111	334	
33	$(CH_2)_n$		160	249	424	
62[d]	**6**	190	100	132	800	522
72[d]	**2,3,5**; *CHO*	(5600)	(2000)	(4530)	(4350)	(3600)
116	unsaturated				570[e]	
130	unsaturated	~1000			495[e]	
172[d]	*COO*	(2300)	(~1100)	(~1200)	680	

[a] From cross-polarization inversion-recovery pulse sequence. Average values, ⟨T_1(C)⟩, are determined from straight-line fits of log (^{13}C signal height) vs recovery time over the range 0.0–0.1 or 0.0–0.25 s. The longer values (in parentheses) are derived from the decay of carbon signals over the time interval 0.25–5.0 s. Estimated uncertainties in the measurements are 10–20%. [b] Polyester carbons are italicized; carbohydrate carbons are in boldface and refer to the structure shown in Table I. [c] From ref 10, where the 30- and 33-ppm resonances correspond to cutin and wax methylenes, respectively. [d] In lime cutin, resonances from oxygenated carbons are found at 64, 73, and 174 ppm. [e] Peaks show biexponential behavior, including slowly relaxing components with time constants of 7.8 s (116 ppm) and 9.9 s (130 ppm). See Figure 4.

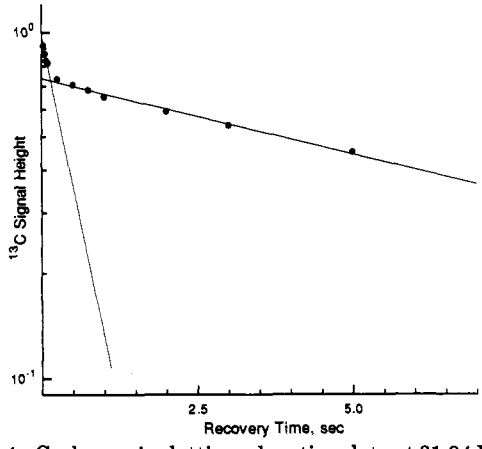


Figure 4. Carbon spin-lattice relaxation data at 31.94 MHz for unsaturated carbons (130 ppm) of potato wound periderm. A least-squares fit of log (^{13}C signal height) vs recovery time over the range 0–0.1 s yields the average carbon spin-lattice relaxation time, ⟨T_1(C)⟩, while a fit of the data for recovery times 0.25–5.0 s yields a value of T_1(C) for the slowly relaxing component. Similar biexponential plots are obtained for the 116-ppm resonance.

alities: the bulk methylenes (30 and 33 ppm) and the phenylpropanoids (116 and 130 ppm). The alkyl-chain carbons are divided into two groups having distinctive chemical shifts, different responses to delayed-decoupling sequences, and different spin-lattice relaxation times. For each phenylpropanoid signal, two different populations are distinguished by their spin-lattice relaxation behavior. The observation of two distinct populations for both aliphatic and unsaturated groups is consistent with the idea that suberin is anchored to the cell wall at discrete sites.[1,7,8] Those portions of the polyester proximal to the attachments display long ⟨T_1(C)⟩'s due to restricted segmental reorientation, whereas distant groups have more motional freedom and hence shorter ⟨T_1(C)⟩'s.

Finally, CPMAS ^{13}C NMR makes it possible to examine the dynamic and structural impact of suberin on the plant cell wall, which is related in turn to the protective function imparted by suberization during the wound-healing response. The spin-relaxation parameter T_1(C) reflects high-frequency dynamic processes, while $T_{1\rho}$(C) and $T_{1\rho}$(H) together probe low-frequency motions. The measurements herein reveal that the carbohydrate segments, already among the most restricted of the functional groups, reorient even less readily at megahertz frequencies after suberin grows within the cell wall. At the same time, all undulations at mid-kilohertz frequencies are *enhanced* by the presence of suberin in the potato cell-wall tissue. Thus it appears that local segmental motions are hampered by the growth of an additional polymer crisscrossing the cell-wall matrix, shifting the distribution of spectral density to lower frequencies and shortening the values of ⟨$T_{1\rho}$(C)⟩. By contrast, both segmental and overall motions are enhanced by the hydrophobic association of cutin and wax chains in lime cuticle.[10]

Despite their differing forms of molecular organization, both suberin-cell wall and cutin-wax assemblies display low-frequency dynamics that suggest the formation of a more resilient barrier layer than either constituent material can form alone. Both the ^{13}C NMR spectra and relaxation behavior displayed by suberized potato cell wall support a model in which the two polymers are attached at discrete sites, possibly by covalent linkages. Using the tools of ^{13}C CPMAS NMR and biosynthetic incorporation of ^{13}C labels during suberin growth, it should be possible to verify these and other chemical transformations that are postulated to protect reactive plant cell-wall groups from pathogenic attack.[2]

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