Magic-Angle Spinning NMR Studies of Cell Wall Bound Aromatic−Aliphatic Biopolyesters Associated with Strengthening of Intercellular Adhesion in Potato (*Solanum tuberosum* L.) Tuber Parenchyma

Bingwu Yu,[†] G. Vengadesan,[‡] Hsin Wang,[†] Liana Jashi,[‡] Tatiana Yefremov,[‡] Shiying Tian,[†] Victor Gaba,[‡] Ilan Shomer,[‡] and Ruth E. Stark*[,†]

College of Staten Island, Department of Chemistry, City University of New York, Graduate Center and Institute for Macromolecular Assemblies, 2800 Victory Boulevard, Staten Island, New York 10314-6600, and Departments of Food Science and Virology, Agricultural Research Organization, The Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel

Received October 26, 2005; Revised Manuscript Received December 7, 2005

Intercellular adhesion strengthening, a phenomenon that compromises the texture and the edible quality of potatoes (*Solanum tuberosum* L.), has been induced reproducibly by exposure to low-pH acetic acid solutions under tissue culture conditions. The resulting parenchyma tissues have been examined by solid-state nuclear magnetic resonance (NMR) in order to characterize the biopolymer(s) thought to be associated with this syndrome. Cross polarization−magic angle spinning (CPMAS) ^{13}C NMR has been used to establish the presence of a polyphenol−suberin-like aromatic−aliphatic polyester within an abundant cell wall polysaccharide matrix in potato tubers that exhibit hardening due to strengthened intercellular adhesion. Dipolar dephasing and CP chemical shift anisotropy experiments suggest that the aromatic domain is composed primarily of guaiacyl and sinapyl groups. Two-dimensional wide-line separation experiments show that the biopolymer associated with parenchyma hardening contains rigid polysaccharide cell walls and mobile aliphatic long-chain fatty acids; ^{1}H spin diffusion experiments show that these flexible aliphatic chains are proximal to both the phenolics and a subpopulation of the cell wall polysaccharides. Finally, high-resolution MAS NMR of parenchyma samples swelled in DMSO in conjunction with two-dimensional through-bond and through-space NMR spectroscopy provides evidence for covalent linkages among the polysaccharide, phenolic, and aliphatic domains of the intercellular adhesion-strengthening biopolymer in potato parenchyma tissue.

Introduction

Environmental stresses such as high temperature, anoxia, and reducing agents promote a strengthening of intercellular adhesion in the parenchyma tissue of plants such as potatoes (*Solanum tuberosum* L.).[1−4] As a consequence of stresses that accompany industrial treatments such as preheating, blanching, peeling, and soaking, the tubers fail to soften as desired during boiling, compromising the edible texture and marketability of commercially processed potatoes produced as flakes, granules, or French fries.[2] This widely studied phenomenon is associated with formation of a stronger middle lamellar complex, which has been attributed in turn to the activity of pectin methyl esterase and subsequent formation of calcium pectate.[5−8] However, this mechanism has recently been questioned (Shomer and Kaaber, submitted), because the enzyme is not optimally active under conditions that promote intercellular adhesion strengthening, and since in contrast to silver-enhanced immunogold localization,[9,10] direct labeling showed the presence of high-methoxyl pectin in both normal and intercellular strengthened tissue.

Because intercellular adhesion strengthening is often induced by environmental stress conditions, it is hypothesized that polymeric secondary metabolites analogous to the suberin formed at the surface of potato wound periderm might be involved. In response to surface stress, the periderm undergoes healing accompanied by assembly of a biopolymer that contains glycerol, phenolic, and aliphatic constituents and is associated with cell wall polysaccharides.[11−15] By contrast, the potato tuber parenchyma interior exhibits stress responses that include necrosis, enzymatic browning with formation of phenolics such as chlorogenic acid, and formation of melanin−protein complexes,[16−18] as cellular rupture generates superoxide radicals and polyphenol oxidase activity.[19,20] Suberin, lignin, and phenolics such as ferulic acid are all thought to play roles in strengthening both the primary cell wall and the middle lamellar pectin.[20−27]

In the current work, intercellular adhesion strengthening in potato tuber parenchyma has been induced reproducibly by exposure to low pH acetic acid solutions under tissue culture conditions. The resulting tissues have been examined by magic angle spinning (MAS) solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy, a powerful and widely used tool for comprehensive elucidation of the chemical structure of amorphous organic solid materials.[28,29] For instance, this method has provided both qualitative and quantitative compositional information on such challenging biomaterials as soil organic matter,[30−34] plant polymers including lignin, cell wall carbohydrates, cutin and suberin,[35,36] and ubiquitous pigments such as melanin.[37] Spin-relaxation measurements offer complementary information on the molecular organization and dynamics

* To whom correspondence should be addressed: stark@mail.csi.cuny.edu.
[†] City University of New York.
[‡] The Volcani Center.

10.1021/bm050812t CCC: $33.50
Published on Web 02/22/2006

of polymer blends,[38] and related 2D wide-line separation (WISE) experiments[39] have been used to deduce site-specific dynamics and domain sizes of biopolymer assemblies. Finally, recently developed high-resolution magic angle spinning (HR-MAS) NMR techniques for solvent-swelled samples[40−45] provide information on both chemical bonding patterns and three-dimensional conformation in the biopolymer(s) associated with "hardening" of the potato parenchyma.

Materials and Methods

Potato Tissue. The induction of intercellular adhesion strengthening ("hardening") was based upon the procedures of Shomer and Kaaber (submitted). Tubers (*Solanum tuberosum* L. cv. Desirée*)* were purchased at a local supermarket in Israel, peeled, surface sterilized (with 1 g/L sodium dichloroisocyanurate "Taharsept"), and washed sequentially five times with sterile distilled water under sterile conditions. Then, the potato tuber parenchyma was excised aseptically into slices (20 × 20 × 0.5 mm^3, ∼2.5 g) that were surface sterilized again for 15 min, washed five times with sterile distilled water and air-dried for 20 min, and finally cultured aseptically in Petri dishes (90 mm) on a solidified mixture containing 20 mL of 50 mM acetic acid buffer (pH 3.5 or 6.5) and 0.8% agar. The cultures were maintained at 25 ± 2 °C in darkness for 5 days. The intercellular adhesion strengthening was then confirmed by texture analysis of both boiled and uncooked tissues using a Stable Micro Systems TA.XT2*i* Texture Analyzer (Surrey, U.K.) in the Volcani Center laboratories, and the samples were freeze-dried.

Removal of Unbound Polysaccharides and Waxes. Freeze-dried potato slices were ground into powder in the U. S. laboratories, using liquid N_2 with a freezer mill (Spex Industries, Edison, NJ). To break down cell wall polysaccharide linkages and release soluble sugars, procedures similar to those applied previously to suberized potato wound periderm[46] were used. (In some preparations, starch granules and cytoplasmic materials were first removed from the cell walls by chopping the tissue slices in a blender and washing repeatedly; the subsequent spectroscopic results were identical.) A 10-g sample of dry potato powder was treated with 100 mL of a 2.5% (w/v) solution of *Aspergillus niger* cellulase (ICN Biochemicals, Aurora, OH; 110 000 units/g) at pH 5.0 in an incubator shaker (New Brunswick Scientific, Edison, NJ) operating at 2000 rpm for 48 h at 37 °C and then 48 h at 44 °C. The resulting insoluble materials were collected by filtration and retreated with cellulase, after which no further decrease in mass was observed. Two treatments were made as follows: with 50 mL of a 6% (v/v) pH 4.0 *Aspergillus niger* pectinase solution (Sigma Chemical, St. Louis, MO; 3000 units/mL) at 28 °C for 24 h; at 31 °C for another 24 h. A final treatment was made with 5 mg/mL hemicellulase (Sigma Chemical, St. Louis, MO; 0.1 units/mg) at 45 °C for 7 days. The filtered insoluble products (∼200 mg) were soaked with stirring for 1 day at room temperature sequentially in hexane, choroform, and methanol and finally washed thoroughly with distilled water. The final material, which amounted to 0.2% of the original dry mass and should be enriched in the putative polymer associated with strengthening of intercellular adhesion, was lyophilized (Virtis Company, Gardiner, NY) for 3 days before use.

NMR Spectroscopy. Solid-state NMR spectra were acquired at The College of Staten Island on a Varian (Palo Alto, CA) UNITY*plus* widebore spectrometer operating at Larmor frequencies of 300.001 MHz for ^1H and 75.443 MHz for ^{13}C. The ^{13}C chemical shifts were referenced to tetramethylsilane via hexamethylbenzene as a secondary substitution reference. Typically, MAS experiments were performed on 50-mg samples of hardened potato parenchyma in a Doty 5-mm XC5 probe (Columbia, SC) operating at room temperature. Other parameters included 5.0-μs pulses for ^1H and ^{13}C, CP contact times of 0.5−2.0 ms unless otherwise noted, and ^1H decoupling strengths of 50−60 kHz. A Varian speed controller was used to maintain typical rotor speeds of 8000 ± 5 Hz, unless otherwise noted. The $^{13}C-^1H$ 2D WISE experiments[47] were performed using pulse sequences that yield ^{13}C chemical shifts and ^1H line widths on two orthogonal frequency axes, while spinning at 3500 ± 5 Hz. The cross-polarization contact time was 500 μs; the spin diffusion mixing time was varied between 0.5 and 20 ms; a zero ^1H frequency offset was used for the $(CH_2)_n$ resonances. Other parameters used in these experiments have been specified previously.[39] Quantitative ^{13}C DPMAS spectra were acquired using a spinning speed of 10 000 ± 5 Hz and a recyle time of 100 s ($>4T_1$s). The dipolar dephasing time in delayed decoupling experiments[48] was 45 μs. A three-pulse chemical shift anisotropy (CSA) dephasing pulse sequence[30,49] was used to quantify the aromatic carbons, with t_{CSA} set to 80 μs. Carbon spin−lattice relaxation times, T_1(C), were measured by monitoring the recovery of ^{13}C magnetization following cross-polarization and inversion.[50] Values of $\langle T_1(C)\rangle$ were determined from the initial rates of this recovery.

MAS ^1H spectra of solvent-swelled hardened potato parenchyma samples were acquired at The College of Staten Island on a Varian ^{UNITY}*INOVA* spectrometer operating at a ^1H frequency of 600 MHz. The latter experiments were performed with a Varian indirect-detection HX nanoprobe equipped with pulsed field gradients, using 2−20 mg of solid, 50 μL DMSO-d_6 at 50 °C, and 3.000 ± 0.001 kHz MAS. Alternatively, spectra were acquired at the New York Structural Biology Center on a Bruker DRX-750 spectrometer (Karlsruhe, Germany) with an inverse-detection high-resolution MAS probe spinning at 10.000 ± 0.001 kHz and equipped with a magic angle gradient. Two-dimensional experiments including gHMQC,[51] gHMBC,[52] tnNOESY,[53;54] and tnTOCSY[55] were conducted using standard pulse sequences. The mixing time was varied from 25 to 500 ms in the NOESY experiments and 10−200 ms for TOCSY experiments.

Results and Discussion

Chemical Composition of Carbon-Containing Materials in Solid Intercellular Adhesion-Strengthened Potato Parenchyma. CPMAS ^{13}C NMR observation of the dry, boiled intercellular adhesion-strengthened ("hardened") potato tissue reveals only the characteristic polysaccharide peaks observed in control samples: resonances at 105 ppm (C-1), 72 ppm (C-2 to C-5), and 60 ppm (C-6). This result indicates, as anticipated in analogy with suberized plant tissues,[56] that at most a small portion of the cultured potato tissue is composed of chemical compounds associated with the hardening phenomenon. To examine these constituents spectroscopically, the "background" of free cell wall polysaccharide was degraded enzymatically to obtain potato parenchyma that was enriched compositionally in any intercellular adhesion-strengthening compound(s).

The resulting CPMAS ^{13}C NMR spectrum derived from potato tissue cultured with pH 3.5 acetic acid (Figure 1, top) shows resonances typical of an aliphatic−aromatic polyester and of the polysaccharides described above: bulk methylenes (30 ppm), oxygenated methylenes (63 ppm) and methines (72 ppm), aromatics and/or olefinics (115−155 ppm), and carboxyl groups (173 ppm). The semiquantitative compositional picture provided by a single CPMAS spectrum shows that polysaccharide signals are still predominant, but there are also substantial numbers of aliphatic chain moieties in evidence (more detailed quantitation is discussed below). By contrast, the control samples (fresh sliced and pH 6.5 acetate) show only signals that are attributable to cell wall polysaccharides. Also notable are spectral differences between the C-1 sugar position in control and hardened tissues: controls display the expected resonances (∼93−108 ppm) from a mixture of polysaccharides present in the cell wall, whereas intercellular adhesion-strengthened materials selectively retain particular sugars (∼100 ppm) that are resistant to the degradative enzyme treatments.

Although the NMR spectrum of the insoluble biopolymer associated with hardened potatoes has smaller aromatic and

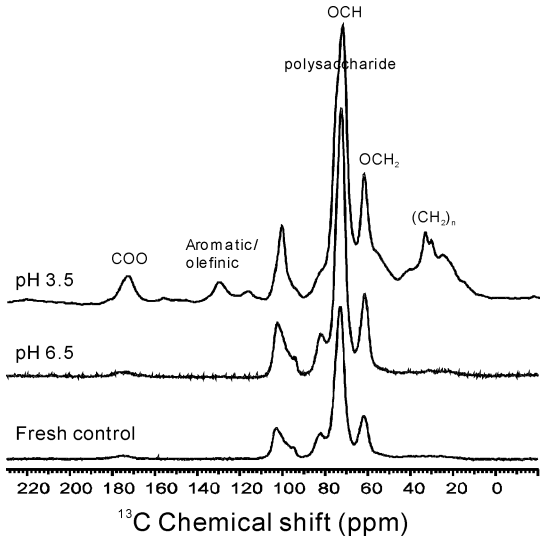

Figure 1. 75 MHz CPMAS ^{13}C NMR spectra of potato parenchyma cultured in 50 mM acetic acid at pH 3.5 (ICA-strengthened); cultured in 50 mM acetic acid at pH 6.5; sliced and analyzed immediately.

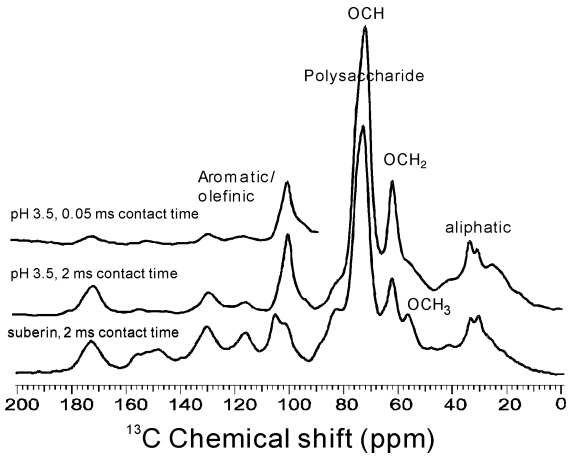

Figure 2. 75 MHz CPMAS ^{13}C NMR spectra of hardened potato parenchyma and suberin from potato wound periderm. The inset shows the spectral region between 100 and 200 ppm acquired with a short contact time, confirming attenuation of signals from nonprotonated carbons due to low CP efficiency.

larger aliphatic peaks than the analogous NMR spectrum of suberized potato wound periderm (Figure 2),[56,57] on the basis of their overall similarities we make the provisional designation of polyphenolic−suberin-like (PS) to describe this material. The solid-state ^{13}C NMR resonances are not in accord with expectations for the common potato phenolic compound chlorogenic acid.[58] The observation of methylene chain resonances at both 30 and 33 ppm may reflect differences in the distance of various chain segments from points of covalent attachment to the cell wall or alternatively variable polyester chain lengths.[57] The identification of the peak at 173 ppm as a carboxyl carbon is confirmed by the significant growth in signal intensity as the contact time is increased from 0.4 to 2 ms in CPMAS experiments (inset to Figure 2) and by its retention in delayed-decoupling experiments (data not shown).

Quantitation of Different Carbon Types in Solid Potato PS. Although CPMAS ^{13}C NMR provides qualitative structural information efficiently for amorphous insoluble polymeric materials, its accuracy in providing the proportions of different domains is compromised by the reduced cross-polarization (CP) efficiency observed for unprotonated or lightly protonated carbons, mobile components, or regions with short values of

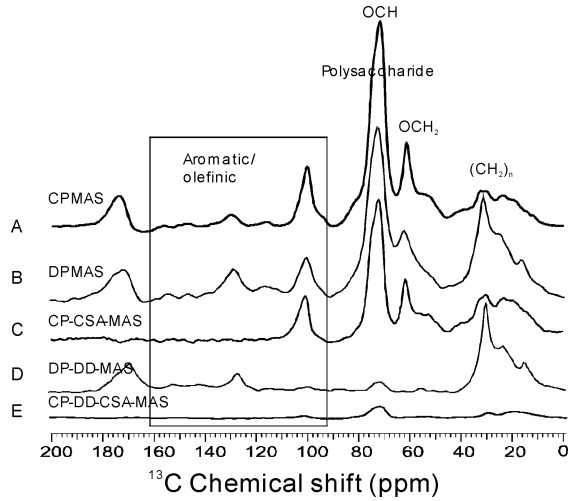

Figure 3. 75 MHz ^{13}C NMR spectra of "hardened" potato parenchyma, plotted with consistent vertical scales to highlight quantitative estimates for the aromatic carbon moieties (90−160 ppm). (A) CPMAS, which may undercount nonprotonated and mobile carbons; (B) DPMAS, providing a quantitative determination of total carbons; (C) CP-CSA-MAS, to selectively suppress aromatic carbons and retain the nonaromatic carbons; (D) DP-DD-MAS, to selectively suppress protonated carbons and retain nonprotonated carbons; the region between 90 and 160 ppm could include aromatic and nonaromatic types; (E) CP-DD-CSA-MAS, to selectively suppress protonated aromatic carbons, leaving a minor residual signal from oxygenated aliphatic (nonprotonated) carbons at 103 ppm. In practice, a small amount of dipolar dephasing occurs for the nonprotonated aromatics, so their intensities in DP-DD-MAS and CP-DD-CSA-MAS spectra are divided by a factor of 0.93.[30] The total aromatic carbons are found from spectra (B) minus (C); the nonprotonated aromatic carbons are found from spectra (D) minus (E). The quantity [(B) − (C)] − [(D) − (E)] then yields the protonated aromatic carbons and the ratio {[(B) − (C)] − [(D) − (E)]}/[(D) − (E)] = (CH/C) is found to be 0.85.

$T_1\rho$(H).[38] Direct polarization (DP) MAS experiments do not have these deficiencies, though they often require very long recycle delays and suffer from poor sensitivity. DP methods are nevertheless practical for many amorphous solids, for which relatively robust molecular motion prevents the necessary acquisition times from becoming prohibitive, and the use of spectral editing schemes (e.g., delayed decoupling (DD), peak suppression for large chemical shift anisotropy (CSA)) permit quantitation of specific functional moieties in challenging biopolymer samples.

Figure 3 compares CPMAS and DPMAS spectra of the same hardened potato parenchyma sample, where the standard spectra displayed in traces A and B are consistent with the expectation that aromatic/olefinic functional groups can be undercounted in CPMAS experiments conducted with a moderate contact time of 2 ms. The bulk methylenes are also underestimated by CPMAS, probably because many of the aliphatic chains are too mobile to undergo effective cross-polarization; similar effects have been reported previously for polyesters in the skin of lime fruits.[59] Correspondingly, the percentage of cell wall polysaccharides, which would impact determination of the domain size from dipolar-filtered WISE experiments, is estimated as 67% using CPMAS but only 53% from DPMAS.

Quantitative estimates made by DPMAS or CPMAS ^{13}C NMR methods are also limited by resonance overlap of the oxygenated alkyl (e.g., ether and sugar) and aromatic/olefinic groups between 90 and 120 ppm. Then, spectral editing experiments that suppress signals from sp and sp^2 carbons that have large chemical shift anisotropies (CP-CSA-MAS) or attached protons (CP-DD-MAS or DP-DD-MAS) can discrimi-

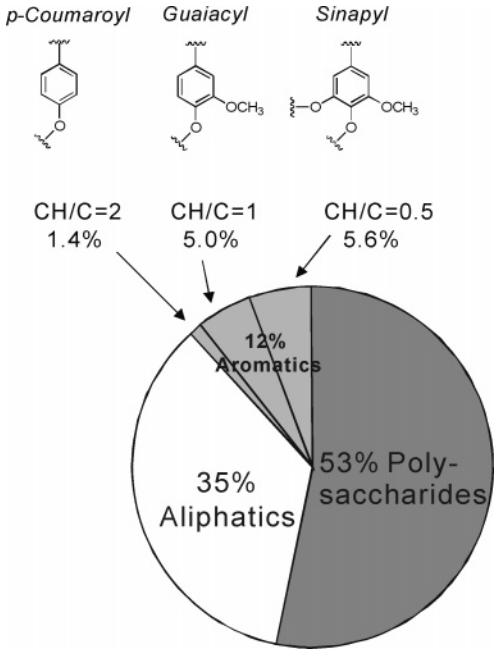

Figure 4. Percentages of different carbon-containing structural moieties in ICA-strengthened potato parenchyma after release of cell wall sugar components, as estimated by solid-state ^{13}C NMR.

nate between aromatic and aliphatic moieties[49] or among aromatic monomer units with different numbers of substitutents,[36] respectively. For instance, Figure 3C (as compared with 3A) illustrates the removal of resonances from carbonyl and aromatic carbons with large chemical shift anisotropies. Figure 3D (as compared with 3B) demonstrates retention of nonprotonated carboxyl and aromatic carbons, along with the expected retention of mobile chain methylenes (see also WISE experiments below) for which dipolar coupling is substantially averaged and dephasing is therefore incomplete during the 45-μs delay before ^1H decoupling begins.[49] The difference spectrum in Figure 3B−C isolates aromatics resonating between 90 and 160 ppm, whereas the corresponding difference spectrum in Figure 3D−E isolates nonprotonated aromatics; the resulting CH/C ratio may then be compared with proposed structures in the parenchyma biopolymers. The final compositional breakdown for hardened potato materials after release of cell wall sugar components is summarized in Figure 4. The aromatic CH/C ratio of 0.85 in intercellular adhesion-strengthened tissue is in reasonable accord with a prior report on suberized wound periderm,[36] supporting a small number of *p*-coumaroyl ring units and predominantly guaiacyl and sinapyl structures that are capable of supporting a densely cross-linked polymeric architecture.

Domain Organization in Solid Intercellular Adhesion-Strengthened Potato Tissue. In addition to revealing the approximate proportions of various aliphatic, aromatic, and polysaccharide moieties, solid-state NMR permits determination of molecular organization in domains within the intercellular adhesion-strengthened tissue. For instance, Figure 5 shows the results of two-dimensional wide-line separation (WISE) experiments[47,60] in which ^1H line widths associated with each carbon site of the ^{13}C CPMAS NMR spectrum are indicative of site-specific mobilities. Although developed initially to study dynamics and domains in synthetic block copolymers or blends, such experiments have also been reported for biopolymers in suberized potato wound periderm[39] and tomato skins.[61] The top set of WISE spectra illustrate different degrees of motional averaging on the 50-kHz time scale: Narrow spectral patterns

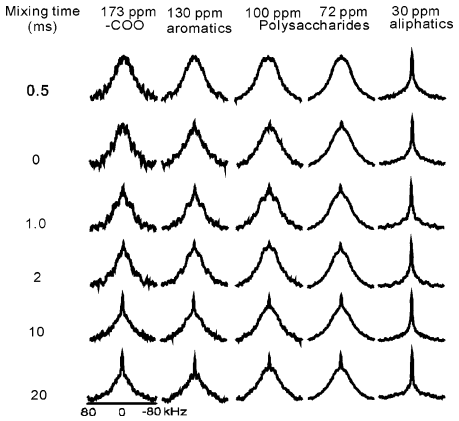

Figure 5. Profiles of motional averaging and domain organization for dry "hardened" potato parenchyma tissue. The patterns show ^1H slices from two-dimensional wide-line separation (WISE) spectra acquired with a proton resonance frequency of 300 MHz and with progressively increasing mixing times as indicated. Line widths are displayed as a function of spin-diffusion time for selected polyester peaks (aliphatics, aromatics, and carboxyls) and for the major polysaccharide resonances.

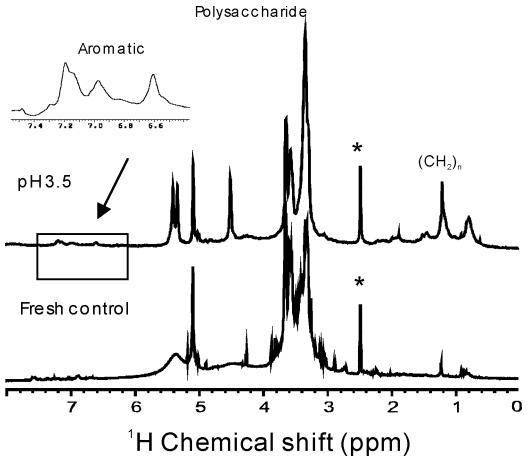

Figure 6. 600 MHz MAS ^1H NMR spectra of DMSO-swelled samples of ICA-strengthened potato parenchyma and a freshly sliced control, obtained with 3000 Hz spinning and a temperature of 50 °C. The * designation refers to residual solvent signals.

(11 kHz) are observed for mobile aliphatic protons bound to long-chain hydrocarbons (30 ppm), whereas broad lines (∼70 kHz) are typical of more rigid polysaccharides, phenolics, and carbonyls (72−173 ppm). This trend is confirmed by measurements of T_1(C) (data not shown), which yield values that range from 4.0 to 28 s for carbons of the aliphatic domain and cell wall carbohydrates, respectively.

If the WISE experiments are performed with various mixing times,[62] the motional narrowing exhibited by the aliphatic chains can spread to spatially proximal functional groups. Figure 5 also shows that, within 0.5 ms, the line widths at half-height decrease by ∼20% for phenolics and carbonyls; in fact, all of the broad peaks rapidly develop a narrow component no more than 3−10 kHz in width. Thus, the progression of spin diffusion suggests that the aliphatic chains of the PS material could be located as close as 0.5 nm from both the phenolics and a subpopulation of the cell wall polysaccharides. As will be seen below, it is possible to test these hypotheses using 2D NMR experiments on solvent-swelled intercellular adhesion-strengthened tissues.

Functional Group Identification in Solvent-Swelled Potato PS. As demonstrated previously for fruit cutins and potato suberin, it is possible to enhance the spectral quality and richness

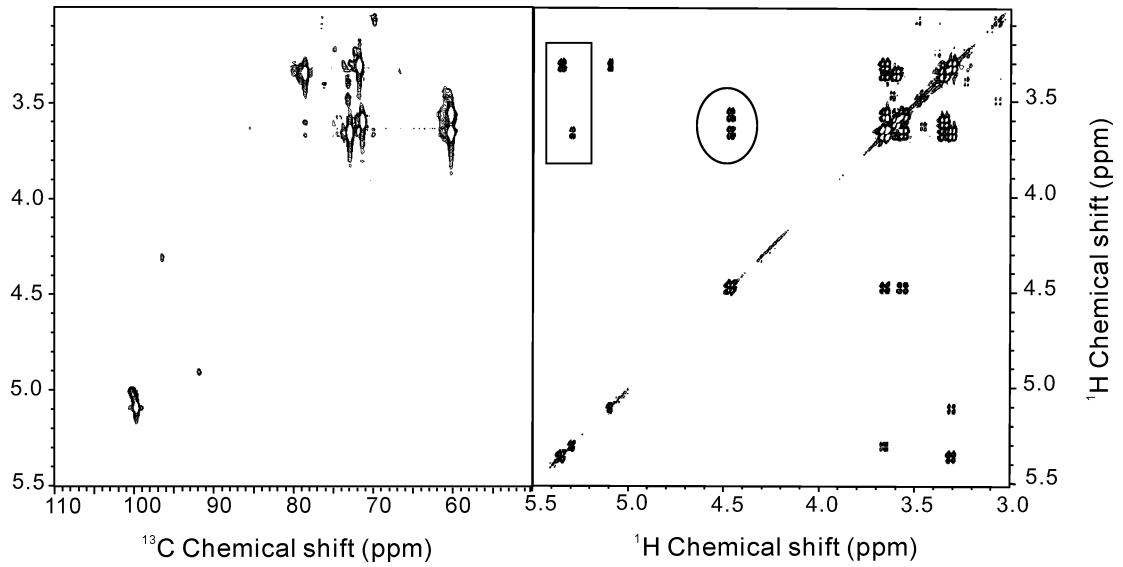

Figure 7. ^{1}H−^{13}C MAS-HMQC (left) and ^{1}H−^{1}H MAS-DQF-COSY (right) spectra of DMSO-swelled hardened potato parenchyma obtained at a proton resonance frequency of 750 MHz. Highlighted COSY cross-peaks show through-bond correlations between hydroxyl and sugar protons, whereas the HMQC correlations confirm the sugar assignments and thus the presence of polysaccharide hydroxyl groups.

of molecular information on potato PS materials, while nevertheless looking directly at solid polymers in preference to small monomeric or oligomeric degradation products.[42,43] Upon swelling in DMSO at slightly elevated temperature, molecular tumbling in the parenchyma tissues described above permits acquisition of high-resolution ^{1}H NMR spectra using MAS at very modest spinning rates. In these experiments, the sample concentration was optimized at 5 mg/50 μL, balancing a large number of nuclear spins (high concentration, up to 20 mg) with an ample accessibility to solvent that produces molecular mobility, long T_2 relaxation times, and persistence of the signal (low concentration, down to 1 mg).

The one-dimensional spectra shown in Figure 6 confirm and refine the compositional information obtained from ^{13}C CPMAS and DPMAS experiments on the analogous dry solids. The ^{1}H NMR spectra of both parenchyma tissue cultured in pH 3.5 acetic acid and of fresh controls show characteristic polysaccharide signals (H-1 at 5.0 ppm; H-2, 3, 4, and 5 at 3.0−4.0 ppm), but only the intercellular adhesion-strengthened materials exhibit additional resonances attributable to PS materials. For instance, the resonances observed between 0.9 and 2.2 ppm confirm the presence of long-chain methylenes and also indicate CH_2 moieties adjacent to carbonyl groups. The signals at 6.6, 7.1, and 7.3 ppm have chemical shifts and scalar couplings (see below) suggestive of a guaicyl moiety. The predominance of aliphatic moieties compared with aromatics that was deduced from DPMAS ^{13}C NMR spectra (Figure 3A) is also confirmed qualitatively in the HRMAS data (Figure 6), but the 8:1 ratio in the latter spectrum likely undercounts the phenolics because of limitations on their molecular motion and MAS-induced spectral narrowing.

The peaks at 4.5, 5.4, and 5.5 ppm in the ^{1}H HR-MAS spectra also exhibit interesting behavior. The 4.5-ppm peak of the pH 3.5 hardened parenchyma tissue disappears totally upon addition of D_2O, suggesting it belongs to hydroxyl group(s) and is likely part of the cell wall polysaccharide matrix. Deuterium exchange also results in a sharp (75%) decrease in intensity for the peaks at 5.4 and 5.5 ppm, though those latter signals do not disappear completely from the NMR spectrum. By comparison, fresh control samples swelled in DMSO display broader resonances than the pH 3.5 parenchyma (Figure 6) that disappear from the HR-MAS spectrum in D_2O (data not shown), consistent with a solvent-accessible polysaccharide structure that permits facile chemical exchange. These trends suggest that the hardened parenchyma tissue has two cell wall polysaccharide types or domains: a highly solvent-accessible structure for which molecular mobility is facilitated and partially hindered sites at which solvent exchange is incomplete and mobility remains limited. As noted above, solid-state 2D WISE experiments also implicate both mobile and rigid polysaccharides; the latter moieties could plausibly correspond to internal cell wall materials that are bound or tightly associated with PS materials.

Table 1. NMR Resonance Assignments for Sugar Signals in ICA-Strengthened Potato Tuber Parenchyma

glucose position	1	2	3	4	5	6
^{1}H shift (ppm)	5.01, 5.09	3.34	3.66	3.31	3.60	3.55, 3.67
^{13}C shift (ppm)	100.0	78.9	73.0	71.9	71.4	60.4

Molecular Architecture of Solvent-Swelled Potato PS. Given the identification of likely aliphatic, aromatic, and sugar-based functional groups, the next logical questions concern if and how these molecular moieties could be linked together covalently. Such a hypothesis has precedent in lignified plant tissues, for which the extraction of lignin−carbohydrate complexes has been presented as evidence for covalent attachment to structural polysaccharides within the plant cell wall.[63,64] Covalent linkages have also been proposed between phenolic and cell wall polysaccharide units in suberized potato tissues, though only indirect spectroscopic evidence involving divergent spin-relaxation behavior and ^{13}C−^{13}C spin diffusion has been reported.[36,57]

For intercellular adhesion-strengthened potato tissues that are swelled efficiently in DMSO,[65] two-dimensional HR-MAS NMR provides a wealth of information that confirms the identification of particular resonances and establishes key structural relationships between their respective structural domains. For instance, the ^{1}H−^{13}C HMQC and ^{1}H−^{1}H COSY spectra (Figure 7) display numerous through-bond connectivities between glycosidic and hydroxyl protons that permit identification of each ring position (Table 1) as well as the sugar hydroxyl groups discussed above (4.46, 5.30, and 5.35 ppm). The absence of a sugar hydroxyl signal corresponding to ring position 2 suggests that this site is in intermediate chemical exchange or

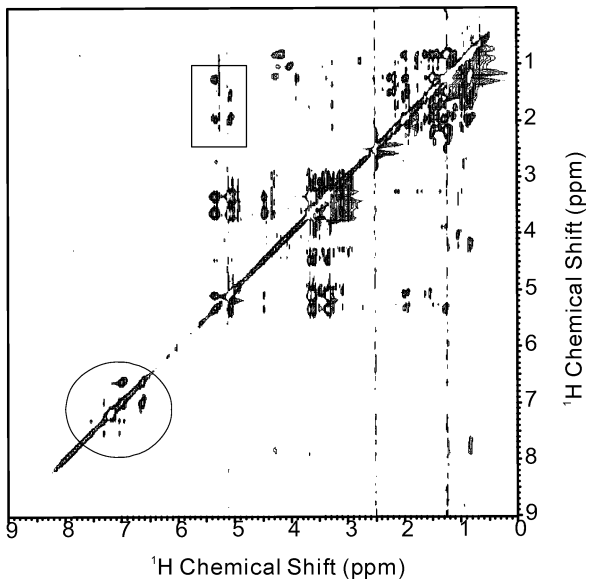

Figure 8. 750 MHz ^{1}H−^{1}H MAS-TOCSY spectrum of DMSO-swelled hardened potato parenchyma, obtained with a mixing time of 100 ms. Highlighted correlations (□) of the aliphatic protons with the sugar anomeric proton, as well as with the sugar hydroxyls, confirm the covalent bonding of aliphatic and polysaccharide domains. In addition, the cross-peak pattern of the aromatic protons reveals the presence of phenolic structures that were not evident in the one-dimensional NMR spectra (○).

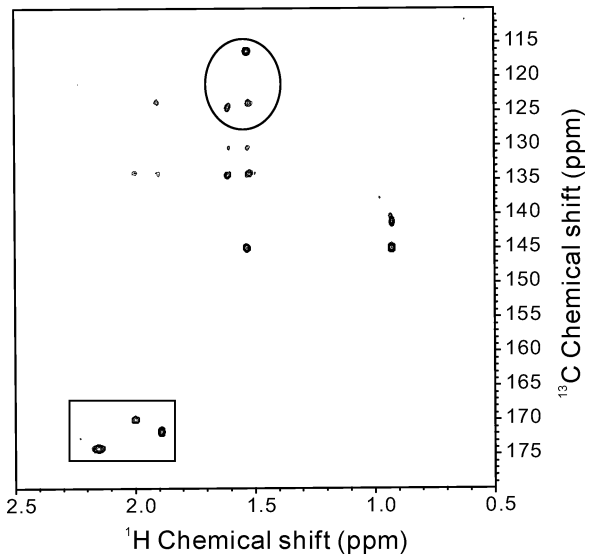

Figure 9. ^{1}H−^{13}C MAS-HMBC NMR of DMSO-swelled hardened potato parenchyma obtained at a proton resonance frequency of 750 MHz. This spectrum confirms the covalent bonding of aliphatic chain and phenolic molecular groupings (○). Three aliphatic carboxyl groups are also identified (□). The cross-peaks at (141.27, 0.93 ppm and 145.02, 0.93 ppm) may be assigned to alkenes; the remaining cross-peaks could be either phenolic−aliphatic linkages or alkenes.

else bonded to a different polymeric constitutent. Larger chemical bonding networks are evident in TOCSY spectra of the swelled PS material acquired with mixing times of 10−200 ms (Figure 8), which exhibit correlations between the anomeric proton of the sugar ring at 5.0 ppm (C-1 at 100 ppm in HMQC) and the aliphatic protons (1.53 and 1.94 ppm). Linkages between the sugar hydroxyls and aliphatic chain protons are also evidenced by TOCSY cross-peaks: 5.30, 1.23; 5.30, 1.94; 5.34, 1.23; 5.34, 1.94 ppm. Thus, some cell wall polysaccharide and aliphatic domains are linked covalently.

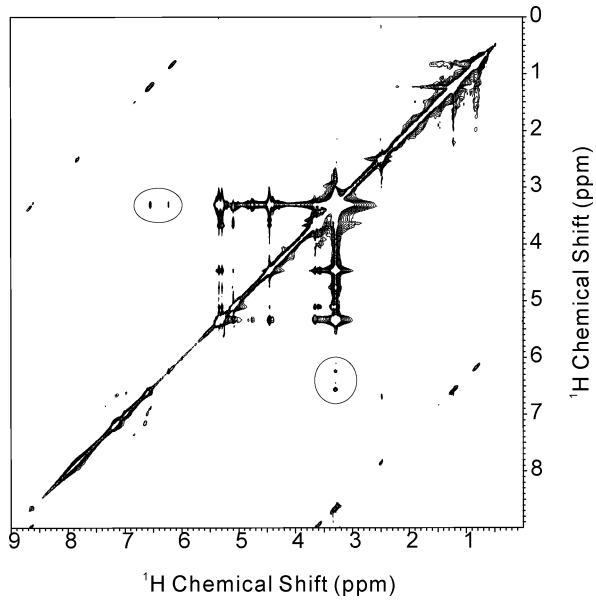

Figure 10. 750 MHz ^{1}H−^{1}H MAS-NOESY spectrum of DMSO-swelled hardened potato parenchyma. Highlighted cross-peaks reveal spatial proximity of polysaccharides to phenolic (○) groups. Assignments of the sugar protons were confirmed from HMQC and COSY experiments, as described in the text. The sets of peaks parallel to the diagonal arise from spinning sidebands.

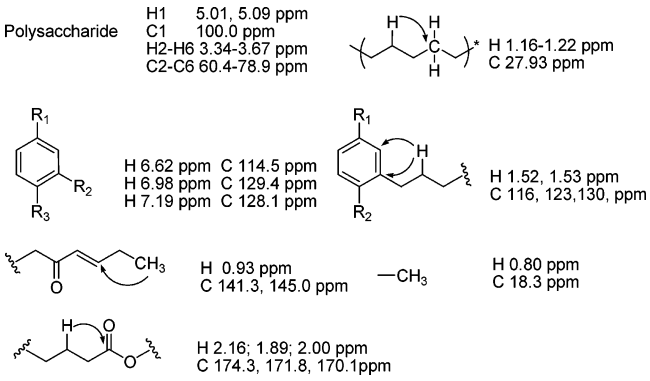

Figure 11. Functional groups found in hardened potato parenchyma using HR-MAS NMR. The arrows denote some of the through-bond connectivities in the MAS-HMBC NMR spectra that provide key support to the proposed structures, as described in the text.

In addition, ^{1}H−^{13}C HMBC spectra (Figure 9) provide potent structural information: cross-peak signatures are observed for three aliphatic carboxylic acid groups (174.3, 2.16; 171.8, 1.89; 170.1, 2.00 ppm), two alkene fragments (145.0, 0.93; 141.3, 0.93 ppm), three phenolic−aliphatic chain linkages (123.7, 1.52; 116.2, 1.53; 123.7, 1.91 ppm), and several linkages of chain methylene protons with carbons that could be attributed to either alkene or aromatic moieties (145.0, 1.53; 134.0, 1.52; 130.3, 1.53; 134.5, 1.61; 130.3, 1.61; 134.2, 1.90; 134.0, 2.00 ppm). Finally, the 2D NMR results of Figure 10 provide evidence for spatial proximity (within ∼0.5 nm) of the phenolic and cell wall polysaccharide groups: NOESY cross-peaks are observed at 6.6, 3.66 ppm and 6.2, 3.66 ppm, where the latter resonance is identified definitively as H-3 of the sugar through its COSY and HMQC connectivities (Table 1). HMBC cross-peaks involving the aromatic structures are quite weak, since as noted above, their signals could be attenuated because of limited molecular mobility. The structural fragments deduced from NMR spectra of the hardened potato parenchyma are summarized in Figure 11.

Conclusions

To gain a molecular-level understanding of stress-related textural defects that compromise the quality of edible plant tissues, NMR methods have been used to analyze the biopolymers associated with intercellular adhesion strengthening in potato parenchyma. Insight into this strengthening phenomenon can guide the design of strategies to improve the marketability of fresh peeled/cut semiprocessed potato products, e.g., by avoiding textural defects associated with stresses that accompany industrial handling and by balancing cell wall integrity with a desirable degree of middle lamellar loosening. By examining these polyesters as intact solids after enzymatic removal of unbound cell wall polysaccharides and using a variety of spectral editing and multidimensional NMR experiments, it has been possible to determine their carbon-containing functional groups, their relative proportions, and many of their interunit linkages. The polyphenol−suberin-like polymer present uniquely in the cell wall of intercellular adhesion-strengthened parenchyma tissues contains long aliphatic chains, cross-linked phenolics, and cell wall polysaccharides that are demonstrated to be both proximal in space and covalently linked to one another.

Although the internal stress responses of potato tuber parenchyma are distinct from the surface healing accompanied by suberin formation, the resulting protective biopolymers may share particular phenolic constituents and undergo covalent bonding to the polysaccharide cell wall. The presence of an aromatic domain in cell walls of intercellular adhesion-strengthened potato tuber parenchyma underlines the role of polyphenols as self-protecting plant metabolites, though the suberin-like phenolics characterized in the present study are distinct from chlorogenic acid, the major phenolic compound found in potato tuber parenchyma. The predominance of guaiacyl and sinapyl monomer units in the PS polymer suggests that, as proposed for potato suberin,[66,67] ferulic and sinapic acids could be biosynthetic precursors in the intercellular adhesion-strengthening process characteristic of potato parenchyma. Additional studies are in progress to delineate and control the biosynthetic pathways for formation of the mature polymers involved in this agriculturally and economically significant phenomenon.

Acknowledgment. We gratefully acknowledge support of this work by the U.S.−Israel Binational Agricultural Research and Development Fund (BARD grant 3368-02) and the U.S. National Science Foundation (grant MCB-0134705). Prof. R.E.S. is a member of the New York Structural Biology Center (NYSBC), a STAR center supported by the New York State Office of Science, Technology, and Academic Research. NMR resources at the NYSBC are supported in part by the National Institutes of Health (grant P41 GM66354). Dr. Hannia Lujan-Upton, Ms. Andrea Phillips, and Mr. Allen Fung assisted with the initial enzymatic treatments and NMR experiments, supported financially by an NSF Research Opportunity Award and NSF Research Experiences for Undergraduates stipends, respectively. Expert NMR assistance was provided by Dr. Boris Itin at the NYSBC.

References and Notes

(1) Stolle-Smits, T.; Beekhuizen, J. G.; Recourt, K.; Voragen, A. G.; van Dijk, C. *J. Agric. Food Chem.* **2000**, *48*, 5269−5277.
(2) Van Dijk, C.; Fischer, M.; Beekhuizen, J. G.; Boeriu, C.; Stolle-Smits, T. *J. Agric. Food Chem.* **2002**, *50*, 5098−5106.
(3) Kaaber, L.; Martinsen, B. K.; Erland Bråthen, E.; Shomer, I. *Lebensm.-Wiss. Technol.* **2002**, *35*, 526−531.
(4) Sapers, G. M.; Miller, R. L. *J. Food Sci.* **1995**, *60*, 762−766.
(5) MacKinnon, I. M.; Jardine, W. G.; O'Kennedy, N.; Renard, C. M.; Jarvis, M. C. *J. Agric. Food Chem.* **2002**, *50*, 342−346.
(6) McMillan, G. P.; Perombelon, M. C. M. *Physiol. Mol. Plant Pathol.* **1995**, *46*, 413−427.
(7) Micheli, F. *Trends Plant Sci.* **2001**, *6*, 414−419.
(8) Stolle-Smits, T.; Beekhuizen, J. G.; Kok, M. T.; Pijnenburg, M.; Recourt, K.; Derksen, J.; Voragen, A. G. *Plant Physiol.* **1999**, *121*, 363−372.
(9) Bush, M. S. *Plant Physiol.* **1999**, *107*, 201−207.
(10) Parker, C. C.; Parker, M. L.; Smith, A. C.; Waldron, K. W. *J. Agric. Food Chem.* **2001**, *49*, 4364−4371.
(11) Fabbri, A. A.; Fanelli, C.; Reverberi, M.; Ricelli, A.; Camera, E.; Urbanelli, S.; Rossini, A.; Picardo, M.; Altamura, M. M. *J. Exp. Bot.* **2000**, *51*, 1267−1275.
(12) Graca, J.; Pereira, H. *J. Agric. Food Chem.* **2000**, *48*, 5476−5483.
(13) Moire, L.; Schmutz, A.; Buchala, A.; Yan, B.; Stark, R. E.; Ryser, U. *Plant Physiol.* **1999**, *119*, 1137−1146.
(14) Parr, A. J.; Ng, A.; Waldron, K. W. *J. Agric. Food Chem.* **1997**, *45*, 2468−2471.
(15) Stark, R. E.; Sohn, W.; Pacchiano, R. A., Jr.; Al Bashir, M.; Garbow, J. R. *Plant Physiol.* **1994**, *104*, 527−533.
(16) Corsini, D. J.; Pavek, J. J.; Dean, B. *Am. Potato J.* **1992**, *69*, 423−435.
(17) Lee, G. S.; Sterrett, S. B.; Henninger, M. R. *Am. Potato J.* **1992**, *69*, 353−362.
(18) Stevens, L. H.; Davelaar, E. *Phytochemistry* **1996**, *42*, 941−947.
(19) Johnson, S. M.; Doherty, S. J.; Croy, R. R. *Plant Physiol.* **2003**, *131*, 1440−1449.
(20) Wallace, G.; Fry, S. C. *Int. Rev. Cytol.* **1994**, *151*, 229−267.
(21) Kato, Y.; Yamanouchi, H.; Hinata, K.; Ohsumi, C.; Hayashi, T. *Plant Physiol.* **1994**, *104*, 147−152.
(22) Montiel, R. K.; Goujon, M.-D.; Jouanin, T. L.; Burlat, V.; Joseleau, J.-P. *Plant Biol.* **2002**, *4*, 2−8.
(23) Ruel, K.; Chabannes, M.; Boudet, A.; Legrand, M.; Joseleau, J. *Phytochemistry* **2001**, *57*, 875−882.
(24) Modafar, C. E.; Tantaoui, A.; Boustani, E. E. *J. Phytopathol.* **2000**, *148*, 405−411.
(25) Mollet, J. C.; Park, S. Y.; Nothnagel, E. A.; Lord, E. M. *Plant Cell* **2000**, *12*, 1737−1750.
(26) Wakabayashi, K.; Hoson, T.; Kamisaka, S. *Plant Physiol.* **1997**, *113*, 967−973.
(27) Waldron, K. W.; Smith, A. C.; Parr, A. J.; Ng, A.; Park, M. L. *Trends Food Sci. Technol.* **1997**, *8*, 213−221.
(28) Schmidt-Rohr, K.; Spiess, H. W. *Multidimensional Solid-State NMR and Polymers*; Academic Press: San Diego, 1994.
(29) Bovey, F. A.; Mirau, P. A. *NMR of Polymers*; Academic Press: San Diego, 1996.
(30) Mao, J. D.; Schmidt-Rohr, K. *Environ. Sci. Technol.* **2004**, *38*, 2680−2684.
(31) Smernik, R. J. *Geoderma* **2005**, *125*, 249−271.
(32) Smernik, R. J.; Oades, J. M. *Eur. J. Soil Sci.* **2001**, *52*, 103−120.
(33) Smernik, R. J.; Oades, J. M. *Geoderma* **2000**, *96*, 101−129.
(34) Smernik, R. J.; Oades, J. M. *Geoderma* **2000**, *96*, 159−171.
(35) Bernards, M. A.; Lewis, N. G. *Phytochemistry* **1998**, *47*, 915−933.
(36) Yan, B.; Stark, R. E. *J. Agric. Food Chem.* **2000**, *48*, 3298−3304.
(37) Tian, S.; Garcia-Rivera, J.; Yan, B.; Casadevall, A.; Stark, R. E. *Biochemistry* **2003**, *42*, 8105−8109.
(38) Schaefer, J.; Stejskal, E. O.; McKay, R. A. *Biochem. Biophys. Res. Commun.* **1979**, *88*, 274−280.
(39) Yan, B.; Stark, R. E. *Macromolecules* **1998**, *31*, 2600−2605.
(40) Deshmukh, A. P.; Simpson, A. J.; Hadad, C. M.; Hatcher, P. G. *Org. Geochem.* **2005**, *36*, 1072−1085.
(41) Deshmukh, A. P.; Simpson, A.; Hatcher, P. G. *Phytochemistry* **2003**, *64*, 1163−1170.
(42) Fang, X.; Qiu, F.; Yan, B.; Wang, H.; Mort, A. J.; Stark, R. E. *Phytochemistry* **2001**, *57*, 1035−1042.
(43) Stark, R. E.; Yan, B.; Ray, A. K.; Chen, Z.; Fang, X.; Garbow, J. R. *Solid State Nucl. Magn. Reson.* **2000**, *16*, 37−45.
(44) Millis, K.; Maas, W. E.; Singer S.; Cory, D. G. *Magn. Reson. Med.* **1997**, *38*, 399−403.
(45) Keifer, P. A.; Baltusis, L.; Rice, D. M.; Tymiak, A. A.; Shoolery, J. N. *J. Magn. Reson.* **1996**, *119A*, 65−75.
(46) Pacchiano, R. A.; Sohn, W.; Chlanda, V. L.; Garbow J. R.; Stark, R. E. *J. Agric. Food Chem.* **1993**, *41*, 78−83.
(47) Schmidt-Rohr, K.; Clauss, J.; Spiess, H. W. *Macromolecules* **1992**, *25*, 3273−3277.
(48) Opella, S. J.; Frey, M. H. *J. Am. Chem. Soc.* **1979**, *101*, 5854−5856.
(49) Mao, J. D.; Schmidt-Rohr, K. *Solid State Nucl. Magn. Reson.* **2004**, *26*, 36−45.
(50) Torchia, D. A. *J. Magn. Reson.* **1978**, *30*, 613−616.

(51) Hurd, R. E.; John, B. K. *J. Magn. Reson.* **1991**, 648−653.
(52) Rinaldi, P. L.; Keifer, P. A. *J. Magn. Reson., Ser. A* **1994**, *108*, 259−262.
(53) Pampel, A.; Kärger, J.; Michel, D. *Chem. Phys. Lett.* **2003**, *379*, 555−561.
(54) Gaede, H. C.; Gawrisch, K. *Biophys. J.* **2003**, *85*, 1734−1740.
(55) Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *65*, 355−360.
(56) Garbow, J. R.; Ferrantello, L. M.; Stark, R. E. *Plant Physiol.* **1989**, *90*, 783−787.
(57) Stark, R. E.; Garbow, J. R. *Macromolecules* **1992**, *25*, 149−154.
(58) Friedman, M. *J. Agric. Food Chem.* **1997**, *45*, 1523−1540.
(59) Zlotnik-Mazori, T.; Stark, R. E. *Macromolecules* **1988**, *21*, 2412−2417.
(60) Clauss, J.; Schmidt-Rohr, K.; Adam, A.; Boeffel, C.; Spiess, H. W. *Macromolecules* **1992**, *25*, 5208−5214.
(61) Round, A. N.; Yan, B.; Dang, S.; Estephan, R.; Stark, R. E.; Batteas, J. D. *Biophys. J.* **2000**, *79*, 2761−2767.
(62) Cai, W. Z.; Schmidt-Rohr, K.; Egger, N.; Gerharz, B.; Spiess, H. W. *Polymer* **1993**, *34*, 267−276.
(63) Ericksson, O.; Goring, D. I.; Lindgren, B. O. *Wood Sci. Technol.* **1980**, *14*, 279.
(64) Wallace, G.; Chesson, A.; Lomax, J. A.; Jarvis, M. C. *Animal Feed Sci. Technol.* **1991**, *32*, 193−199.
(65) Shomer, I.; Vasiliver, R.; Lindner, P. *Carbohydr. Polym.* **1995**, *26*, 55−59.
(66) Bernards, M. A.; Lewis, N. G. *Phytochemistry* **1992**, *31*, 3409−3412.
(67) Arrieta-Baez, D.; Stark, R. E. *Phytochemistry* In press.

BM050812T