Registry No. PVC, 9002-86-2; HCl, 7647-01-0.

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Homopolygalacturonan Nitroxyl Amides: Hydration-Induced Motion

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ABSTRACT: In order to understand the relationship between the degree of polymer hydration, axis of rotation, and rotational frequency as well as rotational anisotropy, we measured the EPR spectra of variably hydrated nitroxyl amide spin-labeled plant homopolygalacturonan (PGA) solids from 77 to 342 K. Detailed spectroscopic simulations using stochastic Liouville theory gave the best fit for experimental spectra assuming a moderate jump model with a rotational anisotropy of 3. The axis of rotation was the magnetic y axis of the nitroxyl amide which corresponds to rotational motion about the polymer's main chain. Internal motions of the dry polymer's nitroxyl ring NH–C bond were found to have two activation energies (0.4 and 3.5 kcal/mol) in the experimental temperature range (77–342 K), indicating a temperature-dependent perturbation of the acid sugar polymer's intermolecular structure. EPR studies of solid matrices with various levels of hydration showed two motional domains corresponding to spin labels with different levels of rotational hindrance. We propose that the "slow" component was due to that fraction of the spin population in highly aggregated helical domains while the "fast" component resulted from isolated blocks of main chain motion due to localized disruption of the ordered matrix. The spin population representing the fast motional domain became the most dominant component of the EPR spectrum upon increasing the temperature and degree of hydration. However, further spectral analysis revealed that the fast component's motion was incompletely averaged and, therefore, was not wholly isotropic.

Introduction

Electron paramagnetic resonance (EPR) has been shown to be an excellent technique to characterize the microstructural and dynamic properties[1–3] of various species. Nitroxyl spin labels have been widely used as probes to obtain information about the nature of localized molecular properties such as conformation, flexibility, and solute–solvent interactions in diverse systems such as poly(phenylacetylene),[4] poly(vinyl alcohol) gels,[5] liquid crystals,[6] vesicles,[7] nucleic acids,[8] lipids, and proteins.[9,10] It has been only within the last several years that a significant amount of work on spin-labeled carbohydrates has appeared in the literature,[11] even less research has been performed on polysaccharides as they exist in their native state. From our laboratory, evidence has been recently presented which indicates that the nitroxyl amine, utilized in this work,[12] covalently attaches through an amide bond to sugar acid polymers in a spatially sequential fashion.

The cell walls[13] of higher plants are roughly analogous to the skeleton of animals. These exceedingly complex, mostly polysaccharide, matrices are biologically important because they modulate cell structure and morphology and act as a primary size exclusion barrier to small molecules as well as pathogenic organisms. One of the most important structural polysaccharides[14,15] of higher plant primary cell wall and middle lamellar complexes are the polygalacturonan-containing macromolecules. The most characteristic physical property of this family of polymers is their ability to form rigid gels[16] and extended arrays[17–19] under aqueous conditions. However, little is understood about the higher order structure[20] of these macromolecules in their natural state (e.g., as a solid). Under such con-

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ditions, hydrogen bonding, hydration effects, and dipolar and ionic forces may interact in complex ways to "fix" these acid sugar polymers in ordered arrays.[21]

In this work, we have chosen the covalently bonded spin label method to study the rotational freedom within homopolygalacturonan matrices as they approach a state of equilibrium hydration, the assumed condition in intact higher plant cell wall/middle lamellar complexes. The dynamical information provided is of interest since the axis of rotation, rotational frequency as well as the degree of rotational anisotropy, and other EPR parameters are affected by the organization and higher order structure of near neighbor homopolygalacturonan species. The molecular structure of a nitroxyl amide labeled monomer unit is shown in Figure 1; x, y, and z are the nitroxyl amide's principal magnetic axes.

Experimental Section

Poly(galacturonic acid) (PGA) and (1-ethyl-3-(dimethylamino)propyl)carbodiimide (EDC) were purchased from Sigma Chemical Co., St Louis, Mo. 4-Amino-2,2,6,6-tetramethylpiperidinyl-1-oxy (4AT) was purchased from Aldrich Chemical Co., Milwaukee, WI.

Spin-Labeled Polymers. The synthesis of nitroxyl amide spin-labeled PGA has been described previously.[12] For this study, the reaction was performed by first dissolving 0.1 g of PGA in 120 mL of distilled water with vigorous agitation. Upon addition of 0.05 g of 4AT (ca. 5×10^{-7} mols) the pH was adjusted to 4.75 by 0.1 N HCl. Aliquots of EDC (<0.1 g) were added to the reaction mixture slowly at 20–30 min intervals to a total of 1 g. The reaction was considered to be complete when the total amount of dilute HCl added was 2–3 mL. Reaction solutions were dialysed exhaustively for 3 days whereupon the samples were lyophilized. Dissolved spin-labeled polymer samples (83 mg/mL) were pipetted into open-ended 2-mm i.d. EPR quartz tubes keeping the length of the solution in the tube ca. 8 mm. These samples were carefully frozen so as to avoid bursting of the tubes during freezing-induced expansion; any samples with a cracked or broken quartz tube were discarded. Following lyophilization, the samples were stored in vacuo over dehydrated silica gel for at least 2 days. The preparation of homopolygalacturonan matrices in this fashion allows for the hydration process to proceed homogeneously due to the greater amount, relative to bulk solids, of channels for gas exchange. For the hydration studies, samples, which had been previously dried in the EPR quartz tubes, were placed in a 100% relative humidity chamber. The level of hydration was monitored gravimetrically as a function of time. Before the EPR measurements, the samples were weighed and immediately put into the liquid nitrogen in order to avoid dehydration. The hydration experiments were duplicated with a deuteriated nitroxyl-labeled PGA sample. The deuteriation was performed by adding 50 mL of ^2H_2O to 100 mg of the sample, stirring, and gentle heating followed for 2 h while flushing with $N_2(g)$. The samples were frozen and lyophilized as described previously.

EPR Measurements. EPR measurements were made with a Varian E-109B X-band spectrometer. (Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned). The amplitude of the magnetic field modulation and microwave power were adjusted so that no line-shape distortion was observable. EPR measurements at 77 K were performed by immersing the samples in $N_2(l)$. The variable-temperature EPR spectra were recorded from 90 to 350 K by using a Varian E-257 variable-temperature accessory. Temperature control was carried out by circulating cold $N_2(g)$, obtained by heating $N_2(l)$, through the EPR cavity via a flow Dewar. The samples were placed in the center of the cavity when a temperature of 90 K was reached; slow heating was performed thereafter to reach the desired condition. The temperature just above the sample was measured by a copper constantan thermocouple while EPR spectra were recorded. The temperature reading error was ±0.5 K. The z-axis component of **A** tensor anisotropy (A_z) was measured by calibration against the 87.3 G hyperfine splitting of dilute Mn^{2+} doped in a MgO matrix. The number of nitroxyl spins was calculated from calibration against a platelike Cr^{3+}/Al_2O_3 crystal (11.38×10^{15} spins).

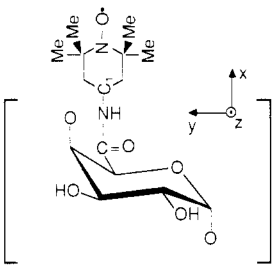

Figure 1. Schematic of nitroxyl spin-labeled poly(galacturonic acid) with the magnetic axes of the label.

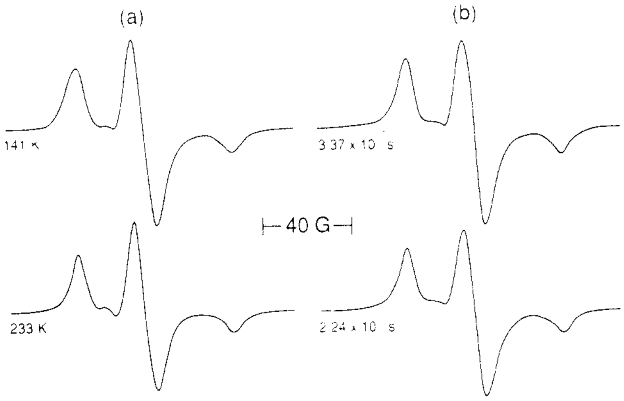

Figure 2. (a) EPR spectra of solid amorphous nitroxyl amide spin-labeled PGA at 141 and 233 K. (b) Simulated spectra of Figure 2a using the slow tumbling program with a rotational anisotropy, $R_{\|}/R_{\perp}$, of 3. The model for rotational reorientation is moderate-jump diffusion. The simulated correlation times are 3.37×10^{-7} and 2.24×10^{-7} s, respectively.

Results

Nitroxyl Amide Motion in the Solid State. The calibration of PGA's spin concentration revealed that there was approximately 0.5 mol % nitroxyl amide indicating that one carboxyl group was labeled for every five to six polygalacturonan chains (the average degree of polymerization has been estimated to be ca. 30–35[19]). This finding, along with relatively small second moments[12] (ca. 525 G^2 at 293 K), suggested that there was no spin–spin broadening contributing significantly to the EPR line shape. The temperature dependence of the EPR spectra of magnetically dilute spin-labeled PGA are shown in Figure 2a. There was a 5.5-G decrease in the outer hyperfine extrema when the sample's temperature was increased from 77 to 450 K, indicating that the nitroxyl amide's motion was sufficient to effect spectral line shape. The spin-labeled macromolecule underwent a fast averaging as evidenced by the rapid decrease of the outer hyperfine extrema at ca. 300 K; the observed line shape was not motionally narrowed at very high temperatures. EPR spectra of the deuteriated nitroxyl amides of PGA exhibited line-shape features similar to the non-deuteriated polymer with the notable exception of a larger outer hyperfine extrema due to a greater z diagonal component of the **A** tensors. The deuteriated nitroxyl-labeled PGA sample did not dissolve well in ^2H_2O, indicating that changes occurred in the hydrogen-bonding properties of the macromolecule upon partial deuteriation of exchangeable hydroxyl functionalities. For the purpose of detailed line-shape analyses[22,23] the non-deuteriated nitroxyl amides of PGA were chosen since the deuteriated sample's $2A_z$ values were quite large; the convergence rate for the simulations in the deuteriated samples would have

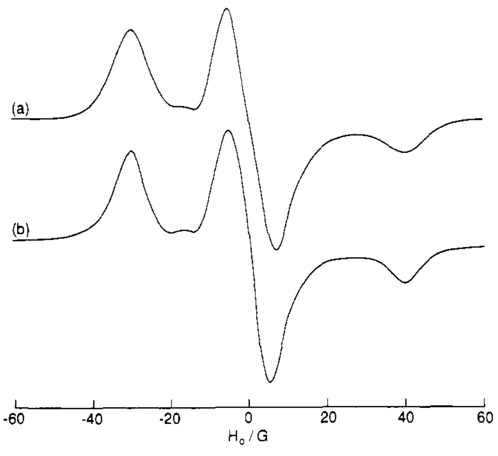

Figure 3. (a) Rigid limit simulation of solid amorphous nitroxyl amide spin-labeled PGA at 77 K. (b) The best fit for a dry non-deuteriated amorphous nitroxyl amide labeled PGA for Figure 3a gave the magnetic parameters: A_x = 7.0 G, A_y = 4.5 G, A_z = 35.8 G; g_x = 2.0095, g_y = 2.0059, g_z = 2.0022; the parameters α and β were 5.8 G and 0.2, respectively.

necessitated excessive computer time.

The experimental spectra measured at 77 K (Figure 3a) were simulated by using a rigid limit program[24] to obtain accurate **g** and **A** tensor components. The rigid limit simulation employs Simpson's numerical integration over θ in 85 intervals and over ϕ in 40 intervals. The spectral parameters, A_z and g_z, can be directly determined from the separation between the outer hyperfine extrema and the midpoint of the two extrema, respectively. The orientationally dependent line width used to fit the spectrum had the form of

$$1/T_2 = \alpha + \beta \cos^2 \theta \qquad (1)$$

where the angle θ is the polar angle relative to the z magnetic axis of the nitroxyl amide (Figure 1). A Lorentzian line shape gave the best fit (Figure 3b) to the experimental EPR spectra. The parameters that gave the best fit were as follows: A_x = 7.0 G, A_y = 4.5 G, A_z = 35.8 G, g_x = 2.0095, g_y = 2.0059, g_z = 2.0022. The orientation-dependent line width parameters, α and β, were 5.8 G and 0.2, respectively.

Detailed spectral simulations ($S = {}^1/_2$ and $I = 1$) were carried out by utilizing stochastic Liouville theory developed by Freed and co-workers,[22,23] the components of the magnetic tensors, **A** and **g**, obtained from the rigid limit simulations, were used as input data. The best simulations (Figure 2b) indicate that the axis of rotation was the magnetic y axis of the nitroxyl amide (Figure 1). Simulations were performed by assuming that the rotational diffusion tensor, **R**, was axially symmetric around the x axis. The best simulations reveal that $|\mathbf{R}|$ along the y axis was 3 times slower than that along either the x or z axes (e.g., the anisotropy of rotation, $R_{\parallel}/R_{\perp}$, = 3). These data were interpreted to indicate that the nitroxyl amide moiety reorients as an ellipsoid form three times faster along x and z than along its longer axis, indicating that the preferred rotation was about the y axis. The magnetic y axis corresponds to PGA's main chain in the molecular frame. The best model for reorientation was a moderate jump. The molecular motion in amorphous solids at low temperatures is generally known to be mainly localized motions possessing low activation energies. In the case of spin-labeled PGA, these detailed simulations demonstrated a local motion which was interpreted as the reorientation of the nitroxyl group at the NH–C$_1$ bond as indicated by the structural representation in Figure 1. The other possible reorientations of the nitroxyl group are comparatively small since the CO–NH coupling has a significant double-bond character; other pyranose ring bond motions seem to be stereochemically unfavorable.

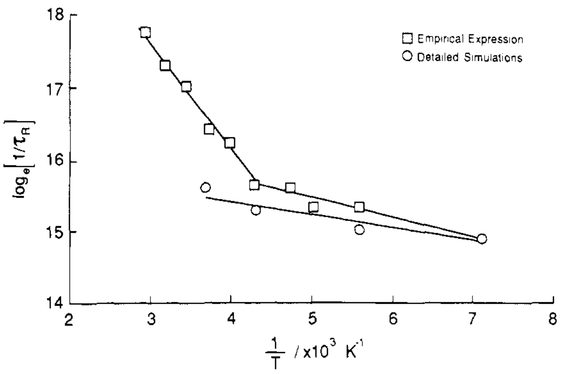

Figure 4. Arrhenius activation energy plots of reorientational motion of nitroxyl amide spin-labeled PGA by using detailed simulations (open circles) from Figure 2b and the empirical expression (eq 2; open squares).

Table I
Parameters, *a* and *b*, and the Calculated Activation Energies (E_a) for Different Models of Rotation[a]

model	line width, G	*a*	*b*	E_a, kcal/mol 179–233 K	 233–342 K
Brownian	0.3	2.57×10^{-10}	–1.78	0.83	3.91
diffusion	3.0	3.40×10^{-9}	–1.36	0.63	5.14
moderate	0.3	6.99×10^{-10}	–1.20	0.55	3.00
jump	3.0	1.10×10^{-9}	–1.01	0.47	3.46
strong	0.3	2.46×10^{-10}	–0.59	0.27	1.69
jump	3.0	3.40×10^{-9}	–0.62	0.28	1.77

[a] SE = ±0.05 kcal/mol.

From the simulation-derived correlation times (τ_R) an activation energy (E_a) of 0.39 kcal/mol (Figure 4, open circles; $\log_e\{1/\tau_R\} = \log_e\{1/\tau_o\} - E_a/RT$) was estimated for the temperature range 141–271 K. For comparison purposes an empirical τ_R (Figure 4, open squares) is also provided; for these calculations, as a first approximation, we have applied the condition that the rotation was isotropic since $R_{\parallel}/R_{\perp}$ was relatively small. Empirical correlation times were calculated as follows[25]

$$\tau_R = a\{1 - S\}^b \qquad (2)$$

whereupon a and b are constants for different models of rotational diffusion and S is the ratio of the separation of the outer extrema ($2A_z$) of a spectrum at a given temperature and that of the rigid limit. The $\log_e (1/\tau_R)$ dependency on reciprocal temperature over the range 179–342 K displayed a transition with two different activation energies (E_a; Figure 4) at 233 K. For each model of rotation, the E_as were calculated (Table I) and were 0.47–0.55 kcal/mol for T = 179–233 K and 3.0–3.6 kcal/mol for T = 233–342 K using the moderate jump model. A comparison of Arrhenius plots of the detailed simulations and the empirical expression (Figure 4) suggests that the E_as, assuming isotropic rotation, were about 15% higher than those from the complete line-shape simulations. X-ray studies[26,27] and conformational calculations[28] on related macromolecules suggest that acid sugar polymer chains can be extensively hydrogen bonded through the carboxyl groups. These observations are consistent with data on the rotational energy between 233 and 342 K since our values are about equal to the energy it takes to form a hydrogen bond assuming that the actual rotation is anisotropic.

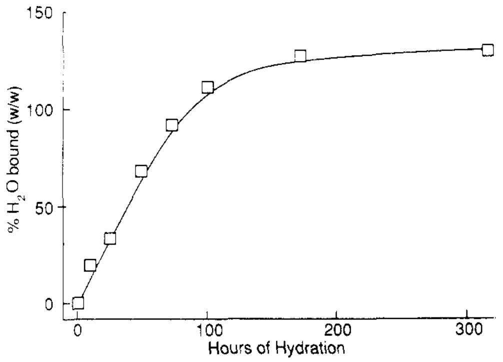

Figure 5. Dependence of polymer hydration on time in a 100% relative humidity chamber.

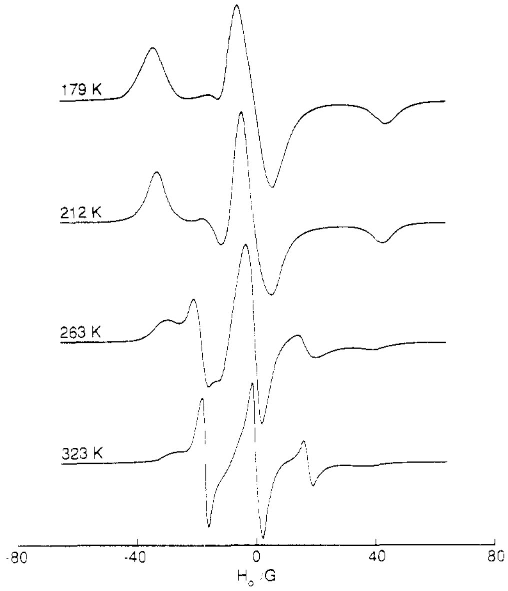

Figure 6. Temperature variable spectra of fully hydrated [100% (w/w)] nitroxyl amide labeled PGA.

Nitroxyl Amide Motion in Hydrated Solids. The adsorption of H_2O onto the spin-labeled polymer matrix is shown in Figure 5 as a function of time in the hydration chamber. As the hydration process approaches a steady state at 100 h, approximately 1 g of H_2O was bound per g of labeled PGA (ca. 10 mol of H_2O/mol of monomer). This degree of hydration is about 30% larger than that reported previously[29] for an intact cell wall/middle lamellar matrix. Samples with a water content of 30% (w/w) are defined, for this work, as moderately hydrated. Any sample with a hydration greater than 50% (w/w) are defined as fully hydrated and contain about equal amounts of bound and free (nonfreezable) H_2O.[30]

We have found that the hydrogen-bonding contribution[31] to the spin Hamiltonian affected $2A_z$ of hydrated samples ca. 3 G more than that of the dehydrated samples. Upon heating the hydrated polymer matrix to approximately 200 K, $2A_z$ decreased drastically as a result of the nitroxyl amide moiety undergoing faster motion. The onset of this narrowing process for a sample with 100% (w/w) bound H_2O is displayed in Figure 6. As the matrix temperature approached 260–270 K, a motionally narrowed, three-line, EPR signal appeared overlapping the anisotropic manifold due to the averaging of the main chain motion around the magnetic x and z axes. The line shape of this apparently isotropic component became sharper with increasing temperature. The appearance of two motionally divergent spin populations at 260–270 K is evidence for two different site geometries; such behaviour was observed in both moderately and fully hydrated samples. Upon saturation hydration, the anisotropic component was still persistent and is evidence that some hindered sites, as in a helical rodlike configuration,[26,27] were stable under these conditions. Detailed line-shape analyses[22,32] have indicated that, if the intensity of the high field peak is not greater than half that of the central peak (e.g., when $T \geq 350$ K), the motionally narrowed component is only incompletely averaged and therefore has some anisotropic characteristics. However, we did not fully analyze $R_{\parallel}/R_{\perp}$ for each component in the composite EPR spectral envelope.

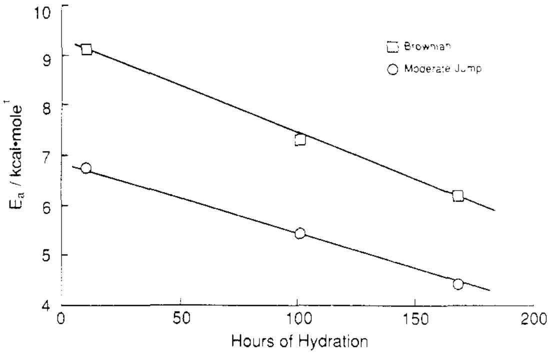

Figure 7. Plot of anisotropic τ_R activation energies as a function of hydration for (open squares) Brownian diffusion and (close squares) moderate jump motional models.

We have compared the τ_R of the nitroxyl amides of PGA in the slow motional domain (200–262 K) by using the empirical expression[25] (eq 2) discussed earlier. The τ_Rs were calculated from the line shape of the dominant, clearly anisotropic, component. The outer hyperfine extrema were used to obtain relative τ_Rs of about 1×10^{-7} s. At room temperature, the nitroxyl amides of PGA show the same spectral features when increasing the level of hydration as with increasing the sample's temperature in that the anisotropic component undergoes a conversion to the fast component as its population decreases with increasing bound H_2O. Inasmuch as there is some error due to the spectral subtraction for the isotropic component, we made an attempt to calculate the τ_Rs from the expressions for the fast motion based on Kivelson theory;[33] we conclude that, even at the highest level of hydration, the nitroxyl amide's motion was not completely averaged.

Slow motional domain τ_R Arrhenius plots gave a preexponential factor on the order of 4×10^8 s^{-1} and a set of E_as ranging from 9.6 to 6.5 kcal/mol as the level of hydration increased to saturation (Figure 7). The range of E_as obtained were consistent with the energy required for local main chain or segmental motion of a few bonds near the nitroxyl amide sites as has been found in other polymer systems.[34] The preexponential factors were in the normal range ($1/\tau_R$ of similar polymers[12] in dilute solution at 293 K were ca. 3.5×10^8 s^{-1}) and not anomalously elevated as in the case of spin probes where values as high as 10^{20} s^{-1} have been reported.[35] The temperature dependence of τ_R displayed true Arrhenius behavior whereupon, for the hydrated PGA matrix, the nitroxyl amide's mobility was dependent on the temperature exclusively and the activation energies were consistent with a segmental motion process.

We also noted that there was a collapse of the apparent free volume of the polymer matrix upon saturation or equilibrium hydration (e.g., as $\nu_1 \rightarrow \nu_{\max}$; the volume fraction of the diluent, ν_1, is approximately equal to degree of hydration in units of g of H_2O/g of PGA). Since the translational diffusion of the penetrant or diluent mole-

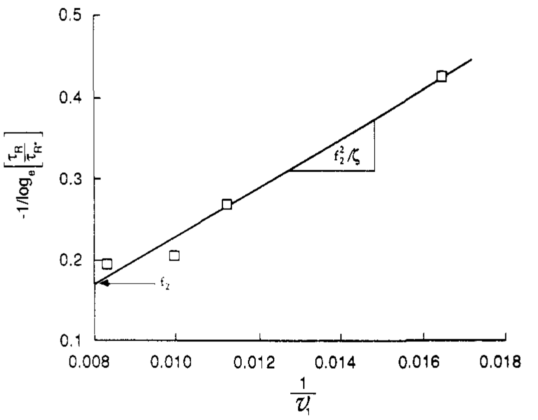

Figure 8. Fujita–Doolittle plot of the isotropic component at room temperature in fully hydrated nitroxyl amide labeled PGA.

cules in certain polymers[36] is related to free volume theory, it is plausible to consider the isotropic rotational diffusion dependence on the diluent in a similar manner. For such a comparison (Figure 8) we have utilized the Fujita–Doolittle equation[36-38] shown below (eq 3). In this relationship, we assume that the fractional free volume associated with the unhydrated polymer (f_2) is approximately equal to Ξ as ν_1 approaches $\nu_{\max}$ or saturation (eq 4; $\tau_{\rm R}$s at satuation and in dilute solution[12] were about equal). In eq 3, $\tau_{\rm R}$s are the rotational correlation times of the isotropic component at room temperature; the reference state ($\tau_{\rm R^*}$) was the dry matrix. For a hydrated solid, ζ is approximately equal to the fractional free volume of the hydrated matrix (f_1) minus f_2. The above equation (eq 3) is based on the assumption that the isotropic rotational diffusion of the nitroxyl amides behave similarly to the translational diffusion of the diluent. Our results are shown in Figure 8. The plot was reasonably linear with $\zeta = 0.09$ which was about 25% smaller than ζs reported for polyisobutylene–n-hexadecane.[36] The parameter, f_2, was calculated from the intercept (at a saturating ν_1s) and was ca. 0.16. At large $[1/\nu_1]$s, the extraction of the fast component line shape was not successful due to blatant inaccuracies; hence, at high diluent concentration (>30% [w/w]) the Fujita–Doolittle equation does fit the EPR data well.

$$\Xi = -\frac{1}{\log_e\left[\tau_{\rm R}/\tau_{\rm R^*}\right]} = f_2 + \left\{\frac{f_2^{\,2}}{\nu_1\zeta}\right\} \tag{3}$$

$$\lim_{\nu_1\to\nu_{\max}} \Xi = f_2 \tag{4}$$

Discussion

Anisotropic Rotation in the Solid State. The internal motion of nitroxyl labels in dry solids at low temperatures has been described for polymers such as poly(phenylacetylene)[32] and polystyrene.[39] Our interpretation that the anisotropic motion is due to the nitroxyl's NH–C$_1$ bond is consistent with these previous findings. The detailed simulations also indicate that reorientation in the dry matrix is about the polymer's main chain axis and supports the rodlike structure proposed previously[26,27] for related macromolecules. We also discovered that an $E_{\rm a}$ transition occurs at 233 K. We feel this transition is associated with the onset of formation of intermolecular hydrogen bonds.[40,41] The 15% discrepancy between the $\tau_{\rm R}$ activation energies obtained from the detailed simulations and those derived from the empirical formula (eq 2) was possibly due to the fact that the actual motion might have been more anisotropic than what the simulated spectra could reveal since the line shape at low temperature is broadened by other inhomogeneous interactions. Possible contributions to such inhomogeneous interactions are the line broadening caused by near neighboring ^{1}Hs as well as possible small differences in nitroxyl group hyperfine interactions on polymer chains with different levels of bound H$^+$ and Na$^+$. In conclusion, we have found that the internal reorientation associated with the NH–C$_1$ bond occurs throughout the whole temperature range, whereas, at high temperatures, the nitroxyl amide forms hydrogen bonds with near neighbor hydroxyl groups.

Hydration Effects on Nitroxyl Amide Motion. The $E_{\rm a}$s that we obtained over the temperature range 200–260 K are in agreement with studies on segmental or local motions in several polymeric systems.[34,42,43] Internal motions with relatively low $E_{\rm a}$s cannot be obtained without doing detailed simulations of each component and superimposing them as has been recently done by Pilar and Labsky.[42] It was found from this study that, in a fully hydrated matrix, the deformed polymer chains underwent segmental motions that were similar to those of polymers in dilute solution. Upon hydration, the nitroxyl amide's motion was dependent upon the polymer–solvent interactions which interrupt the localized intermolecular interactions and cause intramolecular rearrangement of the polymer matrix. The solvent dependence of the isotropic component at room temperature followed sensibly with the Fujita–Doolittle equation suggesting that the rotational motion above 270 K depends on a change in the monomeric friction coefficient and that the homopolygalacturonan segmental chain motions are sensitive to free volume changes in the matrix.

The conversion of the nitroxyl amide's motion from anisotropic to isotropic is evidence for a molecular rearrangement in the polymer matrix. The $E_{\rm a}$ for a helix-random coil transition from kinetic data on a segmented i-carrageenan[44] is about 20 kcal/mol at room temperature. This finding suggests that, as temperature increases, the helical structure proposed for sugar acid polymers is conserved since the activation energies in our study are well under 20 kcal/mol. The aforementioned high $E_{\rm a}$s would be expected for dilute PGA solutions when describing segmental motion whereupon the polymer chains are separated from one another in the form of random coils. Conformational energy calculations[28] suggest that intermolecular forces play a major role in stabilizing helical conformations with threefold symmetry in the solid state. In a hydrated polymer, the intermolecular forces are reduced and the conformations of individual chains are allowed to be expressed. Our data argues that, in the hydrated matrix, the PGA chains still conserve some of the underlying antiparallel sheetlike structure, proposed for the condensed phase of related polymers,[26,27] since the presence of some anisotropic line shape is retained at high temperatures.

The composite EPR line shape seems to indicate an inhomogeneous distribution of the diluent within the matrix representing the two- (or more) site model. In our system we feel that this model is more reasonable in interpreting the diffusion of the water molecules through the polysaccharide matrix than the one-site, or microscopic order with macroscopic disorder, model (MOMD) proposed by Meirovitch and co-workers[45] for fitting the spectra in Figure 6. The previous statement is true because the native helical conformation of these polysaccharides would necessarily tend to force the nitroxyl amide moieties to point out into more than one direction relative to polymer's main axis and therefore be best represented by the two- (or greater-) site model. For the analysis of the two-site model, a bimodal distribution[43] of penetrant has been proposed and extensively discussed by Brown and Sandrezki[46] in describing the distribution of correlation times of nitroxyl probes. However, a broad monomodal distribution[46] of τ_Rs appears to be the best model to describe the nitroxyl behavior in our system. Initially in this model, the absorption occurs in all regions to different levels depending on the degree of intermolecular entanglement whereupon there is a broad distribution of the τ_Rs for the slow and fast components; upon further hydration the distribution moves to the faster τ_Rs or higher frequencies. We have not attempted to mathematically manipulate the data for fitting the exact distribution for the nitroxyl amide correlation times in the hydrated PGA matrix. Of interest is the fact that a Poisson distribution[47] has been shown to describe this selective partitioning of solute molecules between coexisting isotropic and anisotropic phases.

Registry No. PGA, 65290-35-9.

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