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Investigation of Aluminum Binding to a *Datura innoxia* Material Using ^{27}Al NMR

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Al binding to biomaterial derived from cell wall fragments of the plant *Datura innoxia* has been investigated using solid-state ^{27}Al NMR spectroscopy. Carboxylate groups have been determined to be the responsible functionalities for Al^{3+} binding to this material at pH 3.5. The binding of Al^{3+} directly to the polysilicate structure of the immobilized biomaterial was also observed. At pH 5.0, direct binding of the Al_{13}^{7+} polymer ion to the biomaterial was discovered. Carboxylate groups were also determined to be involved in the binding of Al^{3+} at pH 5.0. The presence of an additional octahedral Al-binding site was also suggested at the higher pH.

Introduction

Along with the public awareness of the role of metal ions in the environment, biologically derived materials are attracting increased attentions for their low cost, high selectivity and high capacities in extraction of heavy metals from water systems. Specifically, Al toxicity has recently received scientific and public interest. (*1, 2*) Of particular interest has been its proposed contribution in Alzheimer patients (*2*). A variety of microorganisms including algae, bacteria, fungi, and yeast have been demonstrated to be capable of accumulating different metals from aqueous solutions (*3*−*13*). Certain higher plant tissues and cultured plant cells have also been shown to adsorb metal ions dissolved in aqueous media (*14*−*19*). Previous studies in our laboratory have demonstrated the ability of a *Datura innoxia* cell wall material to remove heavy metal ions from aqueous solutions (*16*−*19*). This material is derived from a plant belonging to the *Solanaceae* family and is native to southwestern United States and northern Mexico and has exhibited a tolerance to toxic metals.

To efficiently apply the *D. innoxia* material for the reclamation and remediation of contaminated water, an understanding of the fundamental chemistry of the metal-binding processes is required. The identification of functionalities involved in the binding process and the chemical form of the bound metal is included in this level of understanding. Such information will enhance the accurate prediction on biomaterial-binding characteristics under real-world conditions, provide guidelines for the selection of optimal binding conditions, and give insight into the possible modification of native biomaterials for improved binding capacity and selectivity.

Previous investigations using Eu(III)-luminescence have indicated that carboxylate and sulfate are the responsible functionalities for the metal ion binding to the *D. innoxia* material (*17, 20*−*23*). Using ^{113}Cd NMR, the involvement of carboxylate groups in the binding of Cd^{2+} was demonstrated for this and other biomaterials (*24*). In this paper, the binding of Al to the *D. innoxia* material will be characterized using solid-state ^{27}Al NMR.

The 27 isotope of Al is a favorable nucleus for direct NMR investigation with a 100% natural abundance and a high receptivity. The relative sensitivity of ^{27}Al is three orders higher than that of the ^{13}C nucleus. Because of the nuclear spin of $^5/_2$, ^{27}Al possesses a quadrupole moment. Its resonances are therefore broadened, especially relative to those of spin $^1/_2$ nuclei. Fortunately, its quadrupole moment is relatively small. This results in relatively high sensitivities. Because of this quadrupole moment, the line width of Al resonance signal is also sensitive to the symmetry and arrangement of ligands about the metal nucleus. The ^{27}Al nucleus exhibits a relatively broad chemical shift window of approximately 450 ppm (*25*). The combination of line width sensitivity and broad chemical shift window make ^{27}Al NMR a powerful probe for studying local metal-binding environments.

Experimental Section

Cultured cell wall fragments from the anther of the plant *D. innoxia* were obtained by procedures described elsewhere (*21*). A portion of this material was subjected to an esterification process to remove any carboxylate groups. This modification procedure has been described in detail elsewhere (*26*). Briefly, a 10.0 g sample of the *D. innoxia* material was soaked in 650 mL of anhydrous methanol (VWR Science, 99.8%). A 5.4 mL volume of concentrated HCl (VWR Science, 36.5−38.9%) was then added to the suspension (0.1 M in HCl). Aliquots of the resulting suspension were then removed periodically over a 3 day period. These samples and the final product were subsequently washed with Nanopure water to remove excess HCl and methanol. Each biomaterial sample was then lyophilized for further study. Infrared absorption spectra have indicated no significant degradation of the cell wall material other than the removal of carboxylate groups by this procedure (*24*).

A portion of the *D. innoxia* cell fragments was immobilized within a polysilicate matrix using a procedure that has also been described elsewhere (*19*). Briefly, a 6% solution of sodium *m*-silicate (Fisher) was added to 300 mL of 5% H_2SO_4 (Mallinckrodt) to adjust the solution to pH 2. Twenty grams of sieved biomaterial (100/200 m) was then added to the mixture. The resulting slurry was vigorously stirred for 1 h. The 6% sodium silicate solution was then slowly added to the slurry until pH 7.0. The rapid formation of a gelatinous polymer was observed. The suspension was stirred for an additional 30 min and allowed to set overnight in a refrigerator. The biomaterial-containing polymer was then washed with Nanopure water until a barium test failed to produce a visible precipitate. The final polymer gel was dried in an oven overnight at approximately 100 °C and subsequently ground to the desired particle size. Pure silicate polymer was also prepared using the same procedure without adding the *D. innoxia* material.

A 0.05 M Al^{3+} stock solution was prepared by dissolution of the sulfate salt (Fisher Scientific Co.) dissolved in 0.1 M MES. The MES buffer was selected because of its demonstrated inability to complex with metal ions (*27, 28*). It should be noted that the MES was used as a means of controling the ionic strength of the solutions rather than to control the pH of the solutions. Concentrated H_2SO_4 and NaOH were used to adjust the pH of solutions to either 3.5 or 5.0. Under these

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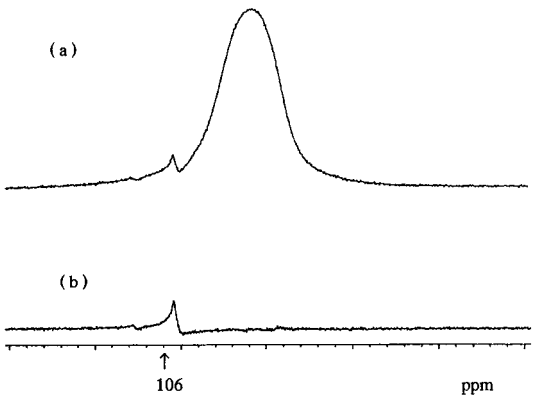

FIGURE 1. Effect of linear prediction. (a) ^{27}Al NMR spectrum of empty sample rotor with normal FT transform. (b) ^{27}Al NMR spectrum of empty sample rotor with linear prediction before FT transform.

conditions, the buffering capacity of each solution was therefore low. A white precipitate was observed when the pH of solution was adjusted to 5.0. The precipitate was separated by centrifugation. Only the resulting clear supernatant was used for the following study. This was subsequently analyzed by ICP emission spectroscopy and fond to have a concentration of 0.033 mM.

A 250 mg sample of either the native or the esterified *D. innoxia* material was suspended in 3.0 mL of the corresponding Al^{3+} stock solution. Similarly 300 mg samples of the immobilized *D. innoxia* material, the Bio-Rex 70 ion-exchange resin, and the pure silicate polymer were each suspended in 2.0 mL of an Al^{3+} stock solution. Each solution was agitated for 1 h. The resulting slurries were each separated by centrifugation, washed with Nanopure water three times, and freeze-dried to achieve a constant mass condition.

To determine the amount of Al bound to each material, 80 mg of each sample was suspended in 3 mL of 1 M HCl, agitated for an hour, and separated with centrifugation. A 1.0 mL aliquot of the supernatant was collected, diluted, and analyzed with inductively coupled plasma (ICP) atomic emission spectrometry (Thermo Jarrell Ash, Atom Comp 61). The initial contact solutions were similarly analyzed.

All NMR experiments were performed on a Varian Unity 400 spectrometer with a 9.4 T narrow-bore superconducting magnet. Liquid-state NMR data were obtained with a 10 mm broad-band probe. Centered inside the 10 mm tubes with each sample was a capillary containing pure D_2O for ^{2}H field frequency lock. Typical experimental parameters were 3.0 μs pulses, 0.10 s acquisition time, 10K Hz weep width with 24K double precision data points, and 10 Hz line broadening.

Solid-state NMR data were obtained with a 5 mm VT CP/MAS probe. A magic angle spinning speed of 7.0 kHz was employed for each analysis. The same experimental parameters as for liquid-state NMR were applied except for a line-broadening parameter of 100 Hz. Backward linear prediction (LP) was used to suppress the probe background signal. The typical LP parameters (VNMR 4.1) were lpfilt = 16, lpnupts = 128, strtlp = 31, strtext = 30, and lpext = 30.

Probe background is a common problem in acquiring ^{27}Al NMR spectra (*29*, *30*). The solid NMR probe and sample rotor used in this study contained appreciable amounts of aluminum metal. The resulting probe background could mask resonances from samples under study. Figure 1a is the MAS ^{27}Al NMR spectrum obtained on the 5 mm VT CP/MAS probe with an empty silicon nitride rotor. This spectrum showed a broad background resonance centering around 15 ppm and a relatively narrow, weak resonance at about 106 ppm.

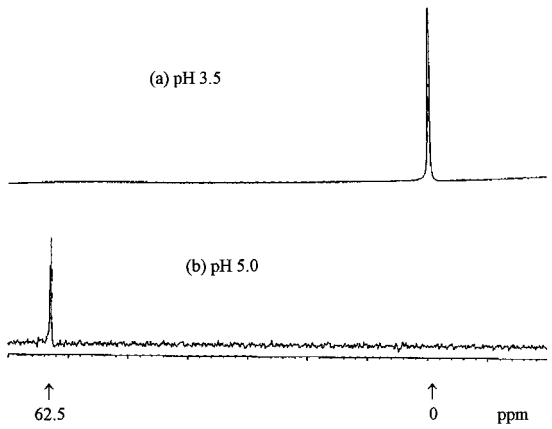

FIGURE 2. ^{27}Al NMR spectra for 0.05 M Al^{3+} contact solutions.

To eliminate the broad background without changing any hardware, backward linear prediction (LP) (*31*) was applied to all solid-state ^{27}Al FID data obtained in this study. Figure 1b is the spectrum based on the same FID data of Figure 1a after a proper treatment of the backward LP. The broad background peak was totally removed while the relatively sharp peak at 106 ppm remained. The 106 ppm signal resulted from the aluminum content of the sample rotor. Because the location and magnitude of this peak was independent of the sample, this signal was subsequently used as a reference for both quantitative and qualitative purposes for all solid-state ^{27}Al NMR spectra. The LP algorithm was therefore applied to all spectra prior to their interpretation as a component of the data collection procedure.

Results and Discussion

Al^{3+}-Contacting Solutions. The hydrated species of aluminum salts depend significantly on the specific solution conditions, specifically pH (*32*, *33*). Metal-contacting solutions at pH 3.5 and 5.0 were used in this study for the binding of Al to the *D. innoxia* materials. Figure 2 shows the ^{27}Al NMR spectra for each of these solutions. At pH 3.5, $Al(H_2O)_6^{3+}$ would be predicted to be the primary species present in solution (*34*). A single resonance at 0 ppm was observed as the only detectable Al species in solution at pH 3.5 and assigned to this species. At pH 5.0, the solution species $[AlO_4Al_{12}(OH)_{24}(H_2O)_{12}]^{7+}$ is predicted to be predominate in solution (*34*). This Al_{13}^{7+} polymer ion consists of a central Al atom which is tetrahedrally coordinated and 12 surrounding Al atoms which are octahedral coordinated (*34*). The central Al is in a highly symmetric environment and gives rise to a characteristic resonance at 62.5 ppm (*34*, *35*). These outer 12 Al atoms of the polymer ion produce largely broadened unobservable resonances (*34*). This resonance shown in Figure 2 for the pH 5.0 contact solution was then assigned to this aqueous species.

^{27}Al NMR Study on Native and Immobilized D. innoxia Material. For the eventual application of biomaterials to the treatment of contaminated water, it is necessary that they be incorporated with some type of rigid support. Biomaterials in their native state usually suffer from poor mechanical strength, low density, and small particle size. They are therefore easily compacted under even mild hydrodynamic pressures (*36*). To investigate the impact of the immobilization procedure on the binding of metal ions, part of the *D. innoxia* material was immobilized within a polysilicate matrix (*19*).

The MAS ^{27}Al NMR spectra of the native and immobilized *D. innoxia* materials after exposure to the Al^{3+}-contacting solution at pH 3.5 are shown in Figure 3, panels a and c. In addition to the reference peak at 106 ppm, the spectrum of the native *D. innoxia* material exhibited only a single

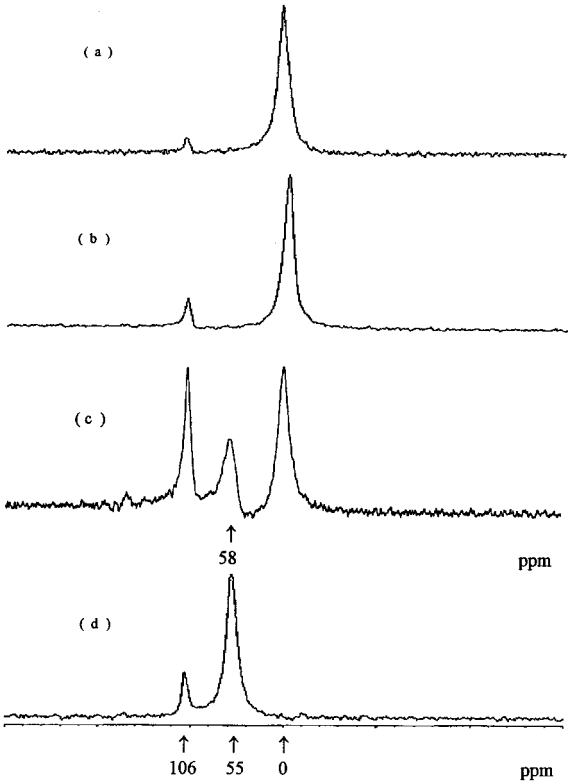

FIGURE 3. MAS ^{27}Al NMR spectra of materials at pH 3.5 (the 106 ppm peak represents same amount of Al in all spectra) (a) Native *D. innoxia* after exposure to 0.05 M Al^{3+}. (b) Carboxylate resin after exposure to 0.05 M Al^{3+}. Immobilized *D. innoxia* after exposure to 0.05 M Al^{3+}. (d) Silicate polymer after exposure to 0.05 M Al^{3+}.

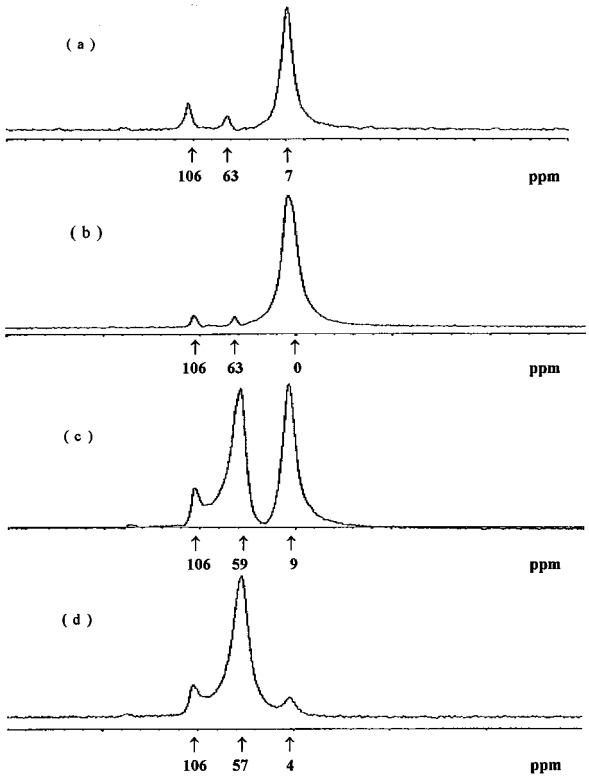

FIGURE 4. MAS ^{27}Al NMR spectra of materials at pH 5.0 (the 106 ppm peak represents same amount of Al in all spectra). (a) Native *D. innoxia* after exposure to 0.05 M Al^{3+}. (b) Carboxylate resin after exposure to 0.05 M Al^{3+}. Immobilized *D. innoxia* after exposure to 0.05 M Al^{3+}. (d) Silicate polymer after exposure to 0.05 M Al^{3+}.

resonance at 0 ppm. In comparison, the spectrum obtained from the immobilized biomaterial showed a resonance at 0 ppm with a second resonance at 58 ppm. Other small spikes observed in Figure 3c were attributed to the presence of spinning sidebands.

Previous studies have demonstrated the involvement of carboxylate functionalities in the metal binding to the *D. innoxia* material (*24*). A resin containing carboxylate groups (Bio-Rex 70) was therefore similarly studied as a model system for the Al binding (Figure 3b). Similar to the native *D. innoxia* material, a resonance at 0 ppm was observed. Assuming that the 0 ppm resonance of both the native and the immobilized *D. innoxia* material was due to the carboxylate-binding sites on the cell wall material of *D. innoxia*, the 58 ppm resonance of immobilized *D. innoxia* was possibly the result of Al^{3+} binding to the polysilicate matrix. A spectrum of pure silicate polymer similarly treated was therefore obtained and is shown in Figure 3d. The observation of a resonance at 55 ppm indicated the involvement of the silicate polymer in Al^{3+} binding.

Al^{3+} binding to the *D. innoxia* material was also studied at pH values of 5.0. At pH 5.0, Al_{13}^{7+} polymer ions were the dominant species in the contacting solution. Figure 4 shows the corresponding spectra of the native *D. innoxia*, the immobilized *D. innoxia*, the carboxylate resin, and the pure silicate polymer after exposure of each material to the 0.05 M Al^{3+} solution at pH 5.0. For the native *D. innoxia* sample, two resonances at 63 and 7 ppm were observed. The characteristic resonance at 62.5 ppm for the central tetrahedrally coordinated Al of the Al_{13}^{7+} polymer has been described by others (*34*, *35*). The relatively narrow peak at 63 ppm in Figure 4a therefore suggests the direct binding of the Al_{13}^{7+} polymer ion to the *D. innoxia* material.

The Al_{13}^{7+} polymer ion was the only species detected by ^{27}Al NMR in the Al-contacting solution at pH 5.0 (Figure 2b). The existence of the 7 ppm resonance therefore suggested the binding of Al to the *D. innoxia* material after decomposition of the polymer ion to the corresponding monomeric species. This 7 ppm chemical shift is downfield shifted relative to that at pH 3.5, suggesting the involvement of functionalities other than carboxylate in the Al binding at pH 5.0. An alternate explanation might be the association of alternate Al species (e.g., one or more of the hydroxyl aquo complexes of the metal ion). However, many of the complexes have lower symmetry which would result in a broadening of the spectral feature. Unfortunately, the relative insensitivity of ^{27}Al chemical shifts to ligand functionalities prohibits the identification of the chemical moieties involved in this alternate binding site.

The ^{27}Al NMR spectrum for the carboxylate resin bound with Al at pH 5.0 is shown in Figure 4b. A 63 ppm resonance was observed in addition to the 0 ppm peak. This also suggests the direct binding of the Al_{13}^{7+} polymer ion with the carboxylate resin. Such an interaction might involve an electrostatic attraction of the positively charged polymer ion to the negatively charged resin (or biomaterial).

As mentioned before, the Al_{13}^{7+} polymer ion consists of one highly symmetrical tetrahedrally coordinated central Al atom and 12 surrounding Al atoms which are octahedral coordinated (*34*). Because of their lower symmetry environments, the 12 outer Al atoms produce largely broadened unobservable resonances (*34*). The integral of the resonance line from the central Al should therefore be multiplied by a factor of 13 to represent the total Al present as the polymer. To verify the binding of Al_{13}^{7+} polymer ion to the *D. innoxia* material, the amount of Al binding to the biomaterial was determined by ICP.

At pH 3.5, one single peak at 0 ppm was observed for the native biomaterial (Figure 3a). Peak area of this resonance was integrated and normalized to the 106 ppm reference

peak. A ratio value of 0.89 was calculated when this normalized peak area was divided by the number of micromoles of Al bound on this material as determined by ICP. At pH 5.0, two peaks at 63 and 7 ppm were observed for the native material (Figure 4a). The sum of these peak areas was calculated and divided by the micromoles of bound Al. A ratio value of 0.42 resulted, significantly lower than 0.89. Because the 63 ppm peak was assigned to the Al_{13}^{7+} polymer ion, This peak area was adjusted by multiplying by 13. With this adjustment, a ratio value of 0.96 was obtained, very similar to the 0.89 obtained for the pH 3.5 sample (Figure 3a). The assignment of the 63 ppm resonance to the bound Al_{13}^{7+} polymer ion was therefore supported.

The immobilized *D. innoxia* material exhibited two Al resonances after exposure to the Al-contacting solution at pH 5.0, one at 59 ppm and the other at 9 ppm (Figure 4c). Similar to the native *D. innoxia* material, the 0 ppm resonance at pH 3.5 was shifted downfield to 9 ppm as the pH was increased to 5.0. This suggests the involvement of functionalities other than carboxylate in this binding site. The 59 ppm resonance was again attributed to the bound Al on the polysilicate structure of the immobilized biomaterial. This assignment is supported by the spectrum of pure silicate polymer after exposure to Al^{3+}-contacting solution at pH 5.0 (Figure 4d). The resonances of bound Al on the polysilicate structure were broad (peak widths at half-height were about 20 ppm). Therefore, if the direct binding of the Al_{13}^{7+} polymer ion to the immobilized biomaterial did exist, the characteristic 62.5 ppm resonance for the central Al of Al_{13}^{7+} polymer ion would be predicted to be buried within these broad peaks.

Chemical Modification. Although the ^{27}Al spectrum of a carboxylate-containing resin suggested the involvement of carboxylates in Al binding, that was not conclusive. Another approach used to verify the involvement of carboxylate groups in metal binding was through chemical modification (*37*, *38*). A Fisher esterification reaction with methanol in the presence of a strong acid was utilized to modify the *D. innoxia* material (*37*, *38*). This reaction can be simply expressed as following

$$R{-}C(O)OH + CH_3OH \overset{H^+}{\rightleftharpoons} R{-}C(O)OCH_3 + H_2O$$

where R represents the organic components of the *D. innoxia*. The successful esterification of those carboxylate groups on the *D. innoxia* material was verified by ^{13}C NMR (*24*). Assuming carboxylate functionalities were involved in the binding of Al, the binding to the esterified material should be significantly reduced.

Figures 5 shows ^{27}Al spectra of the native and the esterified (with different esterification time) *D. innoxia* material. All of these biomaterials had been exposed to the Al^{3+}-contacting solution at pH 3.5. The esterified *D. innoxia* materials (Figure 5, panels b−d) showed largely reduced resonances at 0 ppm than that of the native biomaterial. For the biomaterial which had been esterified for 72 h, the removal of the 0 ppm resonance was nearly complete, strongly suggesting the involvement of carboxylate groups in these 0 ppm Al-binding sites. In addition, the magnitudes of the bound Al on the esterified *D. innoxia* material were observed to decrease as the esterification time increased from 6 to 72 h. The longer esterification time resulted in a greater number of modified sites, thus providing fewer carboxylate groups to be available for metal binding (*38*). This inverse relationship between esterification time and binding capacity is additional evidence for the involvement of carboxylate groups in the Al binding at pH 3.5.

A similar Al-binding study was carried out at pH 5.0 for these esterified biomaterials. Figure 6a shows the spectrum of the native *D. innoxia* material after exposure to the Al^{3+} contacting solution at pH 5.0, while those of the esterified

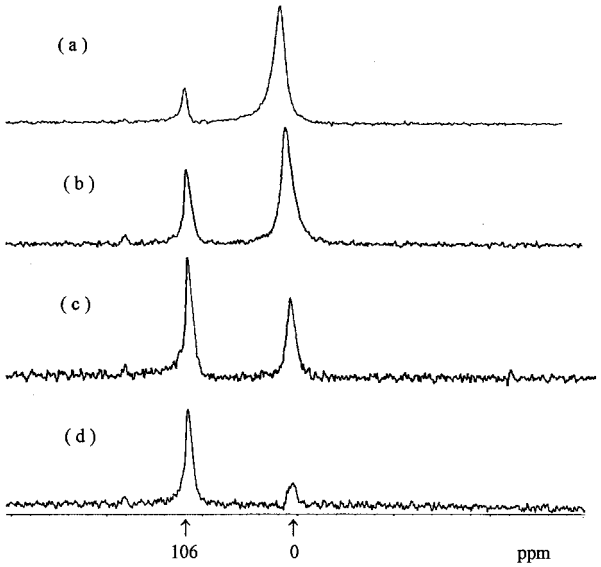

FIGURE 5. MAS ^{27}Al NMR spectra of *D. innoxia* after exposure to 0.05 M Al^{3+} at pH 3.5. (a) Native cell material. (b) Esterification at 6 h. (c) Esterification at 24 h. (d) Esterification at 72 h. The 106 ppm peak represents same amount of Al in all spectra.

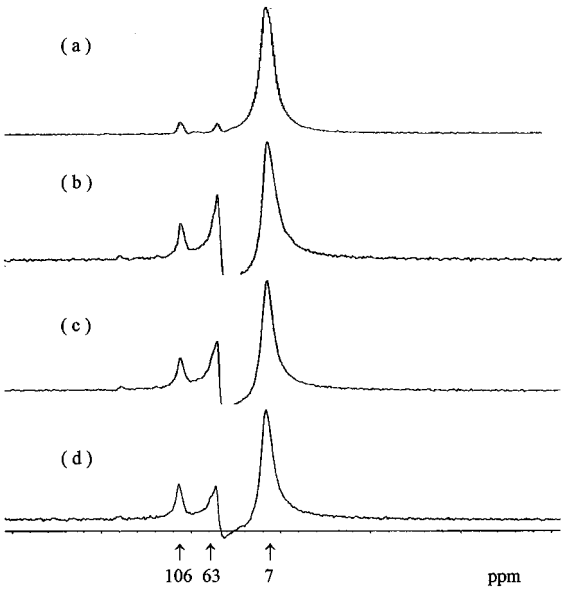

FIGURE 6. MAS ^{27}Al NMR spectra of *D. innoxia* after exposure to 0.05 M Al^{3+} at pH 5. (a) Native *D. innoxia*. Esterification at 6 h. (c) Esterification at 24 h. (d) Esterification at 72 h. The 106 ppm peak represents same amount of Al in all spectra.

D. innoxia (with different esterification time) are shown in Figure 6, panels b−d. The peak distortions observed in Figure 6, panels b−d, were the result of a difficulty in properly phasing the spectrum. Similar to the native *D. innoxia* material, two resonances, at 7 and 63 ppm, were observed for the esterified material. The reduced magnitude of the 7 ppm peak (relative to the internal standard at 106 ppm) following esterification was apparent. This again indicated the involvement of carboxylate groups in these binding sites. In contrast to the pH 3.5 condition, as the esterification time was increased from 6 to 72 h, the 7 ppm peak was not obviously reduced in magnitude. It should be again noted that because the resonance observed at 106 ppm results from trace amounts of Al in the sample rotor, its absolute magnitude is independent of the sample conditions and the relative magnitude of that peak in these spectra is a function of the scaling parameter. From these discussions, it is

therefore suggested that the 7 ppm resonance for the native *D. innoxia* material (Figure 6a) results from two types of binding sites, one with carboxylates and the other involving yet another functionality. The presence of this alternate site could explain the downfield shifted resonance from 0 to 7 ppm when the pH of Al^{3+}-contacting solution increased from 3.5 to 5.0 (Figures 3a and 4a). Because at pH 3.5 the esterification removed almost all of the bound Al at 0 ppm, the second type of binding sites was only available for Al^{3+} binding at higher pH. Conversely, the 63 ppm peak did not reduce in magnitude after the esterification process, suggesting the noninvolvement of carboxylate groups for these binding sites. However, because the binding of such a large ionic species would involved electrostatic forces, alternate anionic functionalities (e.g., sulfate or sulfonate) could be responsible for the binding of this species to the chemically modified biomaterial.

In summary, the involvement of carboxylate groups in the Al binding at both pH 3.0 and 5.0 was concluded. An additional Al-binding site was also suggested at higher pH.

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