Identification of Al(III) Species in a Solution Containing Equimolar Concentrations of Al(III) and Citric Acid Using Varying-Temperature ^{27}Al NMR Spectrometry

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^{27}Al NMR spectra of an equimolar (10 mM) Al(III)-citrate system were obtained at different temperatures. The linewidths of the signals decreased in the spectra recorded at elevated temperatures, which enabled us to unequivocally identify the resonance lines. From the spectrum recorded at 65°C, a mononuclear Al(III)-citrate complex was identified at a solution pH of 3.0 in addition to trinuclear Al(III)-citrate complex, which dominated at pH 4.0.

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Introduction

Aluminum is the most abundant metal in the earth's crust, comprising approximately 7% of the matter in average soil.¹ At neutral pH, it occurs primarily as insoluble deposits that are essentially biologically inactive. In acidified environments, however, aluminum is released as Al(III) ions,² which are inherently toxic to many organisms, and have been identified as neurotoxins.³ Although ambiguity remains, epidemiological studies have implicated Al(III) ions in the etiology or pathology of Alzheimer's disease.⁴ The phytotoxicity of Al(III) ions is also well known,⁵ and is one of the most potent factors limiting crop production in acid soils.⁶ Since Al(III) toxicity depends on the chemical form of the ion, speciation analysis of Al(III) ions is important for an accurate evaluation of metal ion toxicity.

The Al(III) ion is classified as a hard metal ion using the hard acid/soft base criteria.⁷ Therefore, many biomolecules that act as oxygen donor ligands can bind Al(III). Of these, citric acid is the most likely ligand molecule in animals because of its abundance in blood plasma.⁸ This molecule is also implicated in Al(III) tolerance in higher plants.⁹ The formation of Al(III) citrate complexes has been investigated using potentiometric titration analysis,^{10,11} X-ray crystallography,¹² and NMR spectrometry.^{11,12} Here, we report the effect of temperature on ^{27}Al NMR spectra of a solution containing equimolar concentrations of Al(III) and citric acid.

Materials and Methods

Aluminum chloride hexahydrate (Wako Pure Chemicals, Japan) and citric acid monohydrate (Kanto Chemical, Japan) were used without further purification. A solution containing 10 mM Al(III) and 10 mM citrate was prepared at a constant ionic strength of 0.1 M KCl, where 10% D₂O was included as an internal lock. The solution pH was adjusted with 0.1 M HCl and 0.1 M NaOH.

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All the reagents used were of analytical grade. NMR spectra were obtained at least 30 h after sample preparation. The ^{27}Al NMR spectra were recorded on an α -500 NMR spectrometer (JEOL, Tokyo, Japan) at a resonance frequency of 130.2 MHz with a 10.5- μs ($\pi/2$) pulse length, an acquisition time of 150 ms, and a dwelling time of 150 ms. A broadening factor of 5 Hz was applied before the Fourier transformation. The chemical shift was relative to 0 ppm for the resonance of a 10-mM AlCl_3 solution (pH 1.0) at the respective recording temperature.

Results and Discussion

Figure 1 shows the ^{27}Al NMR spectra of a solution containing 10 mM Al(III) and 10 mM citric acid at pH 4.0. When recorded at 25°C, the spectrum showed a broad signal at a chemical shift of 0.2 ppm and another broad signal at 10.8 ppm, with a shoulder near 12 ppm (Fig. 1A). The linewidth of the signals was reduced in spectra measured at higher temperatures (Figs. 1B and C), which enhanced the signal resolution. Three resonance lines were unequivocally identified at chemical shifts of 0.6, 10.9, and 12.9 ppm in the spectra recorded at 45 and 65°C.

A trinuclear Al(III) complex is found in solutions containing Al(III) and citrate.^{10,11} The X-ray crystal structure of the trinuclear anion, $[\text{Al}_3(\text{H}_2\text{L})_3(\text{OH})(\text{H}_2\text{O})]^{4-}$, demonstrates that the three Al(III) ions reside in unequal chemical environments,¹² where H_2L denotes a citric acid molecule with the three carboxylate groups and a fully deprotonated hydroxyl group. The ^{27}Al NMR spectrum of an aqueous solution of $[\text{NH}_4]_4[\text{Al}_3(\text{H}_2\text{L})_3(\text{OH})(\text{H}_2\text{O})]$ crystals has signals at 0.2 and 10.7 ppm, with a shoulder at 12.6 ppm, suggesting that the complex anion is not fluxional in the solution.¹² In addition, each resonance is assigned to one of the three Al(III) ions in the complex anion, using the spectral linewidths.¹² Similar NMR spectra were obtained for a solution containing 0.1 M Al(III) and 0.1 M citric acid at a pH of 7.0.¹¹ Our ^{27}Al NMR study demonstrates that the trinuclear anion is still present at pH 4.0. The Al(III) trinuclear complex is likely the major Al(III) species at this pH, since few signals that could not be attributed to the trinuclear complex were observed. This is consistent with the

Fig. 1 ^{27}Al NMR spectra of a solution containing 10 mM Al(III) and 10 mM citrate at a pH of 4.0. The spectra were recorded at (A) 25, (B) 45, and (C) 65°C.

speciation plot derived from a potentiometric titration analysis.¹⁰

Two factors are associated with the change in the line shape of ^{27}Al NMR spectra of Al(III) complexes recorded at elevated temperatures. An increase in the solution temperature shortens the tumbling time of an Al(III) complex molecule, which produces a narrowed spectrum.¹³ Simultaneously, both the off-rate and on-rate constants of complex formation vary with temperature, and the overall effect tends to broaden the signal due to the decreased residence time of Al(III) in the complex site.¹³ Our finding that elevated temperature reduced the linewidth of the ^{27}Al NMR spectra demonstrates that the major factor determining the linewidth is the molecular tumbling time. Therefore, the residence time of Al(III) on the trinuclear complex is long enough not to affect the line shape. It is also apparent that the structure of the trinuclear Al(III) citrate complex is conserved at temperatures up to 65°C.

Figure 2 shows the ^{27}Al NMR spectra for an equimolar solution of Al(III) and citrate at a pH of 3.0. A sharp signal at 0.1 ppm and broad signals centered on 0.2, and 10.2 ppm were identified in the spectrum measured at 25°C (Fig. 2A). The sharp signal decreased in the spectrum recorded at 45°C (Fig. 2B) and disappeared at 65°C (Fig. 2C). Due to the chemical shift and narrow nature of the signal, the signal was assigned to free Al(III) ions. A decrease in the solution pH should enhance the dissociation of the Al(III) citrate complexes, resulting in free Al(III) ions. The broad signals at 0.2 and 10.2 ppm were narrower at higher temperatures (Figs. 2B and C). In particular, the spectra clearly demonstrated that the broad peak at 10.2 ppm consisted of three signals possessing chemical shifts of 9.4, 10.9, and 12.9 ppm. Therefore, a decrease in the pH from 4.0 to 3.0 results in an emergence of an Al(III) complex with a chemical shift of 9.4 ppm. A shouldered peak at 9.3 ppm was observed in a solution having Al(III):citrate ratios of 1:1, 1:3 and 1:30 with citrate concentration of 0.2 M. The peak may originate from Al(III)HL species coordinated through a single carboxylate.¹⁴ This assignment is based on potentiometric titration data,¹⁵ in which the trinuclear Al(III) complex has not been identified, presumably due to a low Al(III) concentration of 2 mM. Our ^{27}Al NMR study clearly demonstrates the presence of the Al(III) trinuclear complex in an equimolar (10 mM) Al(III)-citrate system. The speciation plot for this system indicates that acidification of the solution from pH 4.0 to 3.0 increases the abundance of a mononuclear Al(III) species, AIL, where the trinuclear complex was taken into account.¹⁰ Here, L represents a citric acid molecule with two donating carboxylate groups and a deprotonated hydroxyl group. Consequently, the AIL complex is the most likely Al(III) species to cause the 9.4 ppm chemical shift. It is unlikely that the equilibrium among

Fig. 2 ^{27}Al NMR spectra of a solution containing 10 mM Al(III) and 10 mM citrate at a pH of 3.0. The spectra were recorded at (A) 25, (B) 45, and (C) 65°C.

the Al(III) complexes has changed considerably, since the spectra showed narrowing only at elevated temperatures.

^{27}Al NMR spectrometry is a powerful tool for identifying Al(III) complexes in solution, since the spectra reflect the chemical milieu around Al(III) ions. However, its application is often hampered by the broadened signal linewidth resulting from quadrupole relaxation, as is the case for NMR spectrometry of nuclei with a nuclear quantum spin number greater than 1/2. One approach to solve this problem is to record the spectrum at an elevated temperature; since the exchange rate of Al(III) is usually slow enough among Al(III) complexes, this leads to a very slow exchange on an NMR timescale. Although caution must be taken to ensure that there is a negligible shift in equilibrium, the method is very simple and should be applicable to complexes with Al(III).

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