Introduction

Aluminium (Al) is a ubiquitous metal ion in the lithosphere, where it is present as an oxide and an aluminosilicate; these compounds are highly insoluble soil minerals. However, when soil acidification occurs through acid deposition, Al is released by the decomposition of soil minerals into the soil solution, and it can mobilize in aquatic environments. The hexaaqua aluminium ion ([Al(H₂O)₆]³⁺, hereafter represented as Al³⁺) and its hydrolytic species exhibit significant toxicity towards plants and animals. The Al toxicity is a serious problem for plants; for example, plant growth and production are strongly limited, and decreased root growth was caused by the presence of the Al species at the micromolar level has been reported. On the other hand, the toxicity of Al significantly depends on its chemical form. Natural organic substances are of great importance for reducing the Al toxicity in acidic soils. Among the numerous Al species, Al-organic acid complexes can effectively reduce the Al toxicity. Therefore, complexation between Al and low-molecular-weight organic acids has been extensively studied because they are ubiquitous in nature, and can form stable complexes with Al. Furthermore, these complexes also play an important role in the Al-tolerance of some plants.

Consequently, the interaction between Al and organic acids is an important research field. During investigations of the complexation between Al and organic acid, dicarboxylic acids were observed to exhibit a strong affinity to Al³⁺. Oxalic acid, which is produced by fungi in forest soils, and is one of the primary components of natural organic acids, is an important chelator of Al³⁺ in forest soils. Oxalic acid can form stable 1:1, 1:2 and 1:3 Al-oxalate complexes in aqueous solutions. Furthermore, in some Al-tolerant plants, the secretion of oxalic acid from the roots is associated with Al detoxification. In addition, Al is accumulated in the cells of leaves as a non-toxic Al-oxalate complex. Low-molecular-weight organic acids represented by oxalic acid clearly play an important role in the detoxification of Al³⁺ in acidified soil. Therefore, a definite determination of complexation between Al and dicarboxylic acid under acidic condition has been required in the research field of Al detoxification.

The liquid-state NMR technique is an effective tool for investigating the complexation of Al in aqueous solutions. Based on NMR measurements and potentiometric titration, the speciation of Al with some organic acids has been investigated. Most of previous studies using NMR techniques have focused on the coordination state of the Al atom in complexes using the ²⁷Al NMR technique. Because many organic acids can interact with Al³⁺ as mono-dentate or bi-dentate ligand, depending on the molar ratio of Al and the organic acid, the peak assignments of ²⁷Al NMR spectra in the previous studies differ from those described in previous papers.

Keywords Al³⁺, dicarboxylic acid, complexation, peak assignment, NMR

(Recieved March 18, 2013; Accepted June 17, 2013; Published August 10, 2013)
the pH and molar ratio, to conduct the further discussion of Al\textsuperscript{3+} complexation, it is also important to investigate the chemical state of carbon atom in each complex. Recently, the interaction between Al\textsuperscript{3+} and simple carboxylic acid has been investigated by \textsuperscript{1}H and \textsuperscript{13}C NMR techniques.\textsuperscript{17–19} These techniques are useful tools to investigate the coordination state and the conformational behavior of these complexes. However, no discussion combining the results of \textsuperscript{27}Al NMR and \textsuperscript{13}C NMR has been reported. Therefore, in this study, we attempted to focus on a quantitative discussion of Al complexes based on the \textsuperscript{13}C and \textsuperscript{27}Al NMR spectra of Al\textsuperscript{3+} and a dicarboxylic acid mixed solution. Especially, we tried to make peak assignments of \textsuperscript{27}Al NMR spectra of Al\textsuperscript{3+} and dicarboxylic acid mixed solution from quantitative discussion. In particular, we conducted all experiments at pH 3 so as to avoid the hydrolysis of Al\textsuperscript{3+}. In addition, this condition is considered to be a limiting pH for soil acidification. We selected oxalic acid, malonic acid and succinic acid as dicarboxylic acids because of the different number of carbon atoms between their two carboxylic groups. We also attempted to compare the effect of the ligand structure on its complexation with Al\textsuperscript{3+}.

**Experimental**

**Reagents and chemicals**

All of the reagents used were of analytical grade. An Al(NO\textsubscript{3})\textsubscript{3} stock solution was prepared by dissolving aluminium nitrate nonahydrate (Al(NO\textsubscript{3})\textsubscript{3}·9H\textsubscript{2}O) in diluted nitric acid (0.1 mol/dm\textsuperscript{3} (M)), and was standardized by EDTA titration. Oxalic acid dehydrate (C\textsubscript{2}H\textsubscript{2}O\textsubscript{4}·2H\textsubscript{2}O), malonic acid (C\textsubscript{2}H\textsubscript{4}O\textsubscript{4}) and succinic acid (C\textsubscript{4}H\textsubscript{6}O\textsubscript{4}) were commercially purchased (Wako Pure Chemical Industries, Ltd.) and was used without further purification. All solutions were prepared using deionized-distilled water.

**Preparation of sample solutions containing each dicarboxylic acid**

Sample solutions were prepared by mixing Al stock solution with the respective organic acid stock solution in the required molar ratios. The Al concentrations in the mixed solutions for measurements of \textsuperscript{27}Al NMR and \textsuperscript{13}C NMR were 0.01 and 0.05 M to 0.2 M, respectively. The sample solution was allowed to stand for 24 h before the NMR measurement. With the exception of the oxalic acid system, the pH of the solutions was adjusted by adding a sodium hydroxide solution. Because the solubility of sodium oxalate is relatively low, precipitation might occur when the pH of the solution is adjusted. Therefore, a potassium hydroxide solution was used to adjust the pH in the oxalic acid system.

**\textsuperscript{13}C and \textsuperscript{27}Al NMR measurements**

The \textsuperscript{27}Al NMR spectra were recorded on a JEOL GX 400 spectrometer operated at 104.2 MHz (pulse width, 20 μs; flip angle, 90°; spectral width, 20000 Hz; sampling points, 8192; acquisition time, 0.328 s; pulse delay, 0.07 s and 512 transients). Each sample solution was placed in a 10-mm Teflon tube. A D\textsubscript{2}O solution enclosed in a Teflon tube with a diameter of 2.5 mm was placed into the 10-mm Teflon tube. All chemical shifts were relative to the external Al(H\textsubscript{2}O\textsubscript{6})\textsuperscript{3+}. The \textsuperscript{13}C NMR spectra were recorded by two measurement condition. The \textsuperscript{13}C NMR spectra were recorded on a JEOL GSX 400 spectrometer operated at 100.5 MHz (pulse width, 4.7 μs; flip angle, 45°; spectral width, 27000 Hz; sampling points, 32768; pulse delay, 1.4 s; 10000 or 13000 transients). Above all, the \textsuperscript{13}C NMR spectra which conducted the quantitative discussion from integration were also recorded by following measurement condition (pulse width, 10 μs; flip angle, 90°; spectral width, 27000 Hz; sampling points, 131072; pulse delay, 15 s and 2000 or 4000 transients). For quantitative integration, the experimental condition which the peak intensity due to carboxylic group is constant was determined by changing pulse intervals in preliminary experiments. Each sample solution was placed in a glass tube with a diameter of 5 mm. A D\textsubscript{2}O solution that contained sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) enclosed in a glass tube with a diameter of 2.5 mm in diameter was placed in the 5 mm tube as an external standard. The chemical shifts were relative to TMS by using the DSS as a secondary reference. The NMR measurements were performed at ambient temperature.

**Results and Discussion**

**Interaction between aluminium ion and oxalic acid**

The interaction between Al\textsuperscript{3+} and oxalic acid (OX) had been previously investigated through potentiometric titrations and NMR measurements. In the potentiometric studies, the presence of Al(OX)\textsuperscript{2+}, Al(OX)\textsuperscript{3+} and AlH(OX)\textsuperscript{2+} had been suggested.\textsuperscript{13,16} In the \textsuperscript{27}Al NMR studies, Thomas et al. assigned that the peaks at 5.9 and 12.2 ppm in the \textsuperscript{27}Al NMR spectrum are due to the 1:1 bi-dentate complex (Al(OX)\textsuperscript{2+}) and the 1:2 bi-dentate complex (Al(OX)\textsuperscript{3+}), respectively.\textsuperscript{21}

Figure 1 presents the \textsuperscript{27}Al NMR spectra of mixed solutions of aluminium ion (Al\textsuperscript{3+}) and OX at pH 3 with various OX/Al molar ratios. The total Al\textsuperscript{3+} concentration is 0.01 mol/dm\textsuperscript{3}.
Figure 2 presents the $^{13}$C NMR spectra for sample solutions with a pH 3 having various molar ratios of OX/Al. The total Al concentration in the sample solutions was 0.05 M. The peak at 169.2 ppm is due to the carboxyl group in free OX, and the peaks at approximately 0 to 58 ppm are due to the DSS. For mixed solutions having OX/Al molar ratios of 1 and 2, the chemical shift corresponding to free OX was not observed, and a sharp peak at 168.0 ppm was observed. The peak at 168.0 ppm can also be assigned to the carboxyl group in the Al-OX complex. Bodor et al. reported in detail on the peak assignments at 168.0 ppm of the $^{13}$C NMR spectrum of Al and the OX mixed solution.17 They assigned the signals at 168.04, 168.10, 168.16 and 168.26 ppm to the Al(OX)$^+$, trans-Al(OX)$^-$$^-$, cis-Al(OX)$^-$$^-$ and Al(OX)$^2-$, respectively. Therefore, this peak at 168.0 ppm in our $^{13}$C NMR spectra is considered to contain these complexes. Because only the peak at 168.0 ppm was observed, all of the OX molecules interact with the Al$^{3+}$ in sample solutions with OX/Al ratios of 1 and 2. In addition, only a peak at 168.0 ppm was observed, which indicates that all of the OX combined with Al$^{3+}$ as a bi-dentate ligand. In the solutions with OX/Al of 3 and 4, peaks due to both the complex (168.0 ppm) and free OX (168.2 ppm) were observed. From the total OX concentration in the sample solution and the peak area ratio of the two peaks at 168.0 and 169.2 ppm in the quantitative $^{13}$C NMR spectrum of the sample solution with an OX/Al = 4, the chemical structure and composition of the Al-OX complex were determined. The intensity ratio of free OX and OX of the complex is 10.8:28.8 (approximately 1:3). In the $^{27}$Al NMR spectra of the sample solution with the same OX/Al molar ratio (OX/Al = 4) (see Fig. 1), the dominant peak is that at 16 ppm. Therefore, from the integration ratio in the $^{13}$C NMR and the $^{27}$Al NMR spectra, the peak at 16 ppm may be assigned to the Al(OX)$^2-$ complex.

To further investigate the complication between Al$^{3+}$ and OX, the $^{27}$Al NMR spectra were deconvoluted. An example of the deconvoluted spectrum is shown in Supporting Information, Fig. S-1. The area ratio and calculated Al concentration of the two peaks at 16 and 12 ppm in the $^{27}$Al NMR spectra that are shown in Fig. 1 are presented in Supporting Information, Table S-1. Using the peak area ratio and the total Al concentration (0.01 M), if we can assume that the two peaks at 16 and 12 ppm originated from Al(OX)$^2-$ and Al(OX)$^-$$^-$, respectively, the experimental and the calculated total Al concentrations are not in agreement (see Supporting Information, Table S-2). Therefore, another hypothesis for the assignment of the two peaks at 12 and 16 ppm is required. To conduct further discussion, we formulated two hypotheses about the peak assignments of the $^{27}$Al NMR spectrum. One hypothesis is that both of the two species (Al(OX)$^2-$ and Al(OX)$^-$$^-$) correspond to the peak at 16 ppm in the $^{27}$Al NMR spectrum. Another is that the existing ratio of each complex in the mixed solution depends on the OX/Al ratio of the mixed solution. In other words, we assumed that the amount of Al(OX)$^2-$ increases with increasing OX/Al molar ratio in the sample solution. When we calculated based on these hypotheses, the calculated and experimental Al and OX concentrations were in agreement (the result of calculation are summarized in Supporting Information, Table S-3). Based on this result, the distribution of Al(OX)$^2-$ and Al(OX)$^-$$^-$ with various OX/Al molar ratios is shown in Fig. 3. As the OX/Al molar ratio was increased, the amount of Al(OX)$^2-$ increased. In addition, because the peak at 16 ppm originated from Al(OX)$^2-$ and Al(OX)$^-$$^-$, the peak at 12 ppm in the $^{27}$Al NMR spectra can be assigned to Al(OX)$^-$$^-$.

To determine the chemical species at 6 ppm in the $^{27}$Al NMR spectrum, the $^{27}$Al NMR spectrum of the solution with an OX/Al molar ratio of 1.0, which is presented in Fig. 1, was deconvoluted. The deconvoluted spectrum of the mixed solution with an OX/Al molar ratio of 1.0 is shown in Supporting Information, Fig. S-2. The peak area ratios of 16, 12 and 6 ppm are 1, 12.55 and 2.32, respectively. At this OX/Al molar ratio, the chemical species at 16 ppm is Al(OX)$^-$, and that at 12 ppm is Al(OX)$^+$ (see Fig. 3). The calculated Al and OX concentrations that correspond to the peak at 6 ppm calculated from total Al concentration and peak area ratio are 1.461 × 10$^{-3}$ M and 8.301 × 10$^{-4}$ M, respectively. Therefore, when the chemical species at 6 ppm is determined using the peak area ratio of the deconvoluted $^{27}$Al NMR spectrum and the total Al concentration, the peak at 6 ppm can be assigned to the Al$_3$(OX)$_4$ complex. At an OX/Al ratio of 0.4, only the peak at 6 ppm in addition to the [Al(H$_2$O)$_6$]$^{3+}$ was observed in the $^{27}$Al NMR spectrum. Furthermore, in the $^{13}$C NMR spectrum, only a new peak at 167.2 ppm was observed, which indicates that all of the OX combined with Al$^{3+}$. This peak is due to the OX that combined with Al$^{3+}$ as a monodentate ligand (Al$_2$(OX)$^{3+}$).

For the Al-OX complexes, the existence of a monodentate complex (AlH(OX)$^{2+}$) at pH 1.5 to 2 has been reported.14 In this study, all experiments were conducted at pH 3. Therefore, we assumed that the AlH(OX)$^{2+}$ complex is not present in the sample solution. The distribution of Al-OX complexes at pH 3 with various OX/Al molar ratios was summarized in Fig. 4.
concentrations for each complex are summarized in Supporting Information, Table S-4. In the OX system, we could propose a new interpretation of the peak assignments of the $^{27}$Al NMR spectra based on a quantitative discussion concerning complementary measurements of $^{13}$C and $^{27}$Al NMR spectra.

**Interaction between aluminium ion and malonic acid**

In potentiometric investigations of the complexation between $\text{Al}^{3+}$ and malonic acid (MA), the formation of Al(MA)$^{+}$, Al(MA)$_2$ and Al(MA)$_3$ has been reported.$^{19}$ In the $^{27}$Al NMR spectrum, the peak at 2.2 ppm can be assigned to the Al(MA)$_3$ complex.$^{16}$

Figure 5 presents the $^{27}$Al NMR spectra for sample solutions with pH 3 having various ligand (MA)/Al molar ratios. The total Al concentration in the sample solution was 0.01 M. The broad peak at approximately 2 ppm can be assigned to the Al-MA complex. With MA/Al molar ratios greater than 2, only the peak at approximately 2 ppm was observed, which suggests that all of the Al$^{3+}$ interacted with the MA. Figure 6 presents the $^{13}$C NMR spectra for sample solutions with pH 3 which contained various MA/Al molar ratios. Figure 6(a) is

![Species distribution curves in the Al$^{3+}$ and oxalic acid system at pH 3.](image)

**Fig. 4** Species distribution curves in the Al$^{3+}$ and oxalic acid system at pH 3.

![27Al NMR spectra of mixed solutions of aluminium ion (Al$^{3+}$) and MA at pH 3 with various MA/Al molar ratios. The total Al$^{3+}$ concentration is 0.01 mol/dm$^3$.](image)

**Fig. 5** $^{27}$Al NMR spectra of mixed solutions of aluminium ion (Al$^{3+}$) and MA at pH 3 with various MA/Al molar ratios. The total Al$^{3+}$ concentration is 0.01 mol/dm$^3$.

![13C NMR spectra of mixed solutions of aluminium ion (Al$^{3+}$) and MA at pH 3 with various MA/Al molar ratios. The total Al$^{3+}$ concentration is 0.05 to 0.2 mol/dm$^3$.](image)

**Fig. 6** $^{13}$C NMR spectra of mixed solutions of aluminium ion (Al$^{3+}$) and MA at pH 3 with various MA/Al molar ratios. The total Al$^{3+}$ concentration is 0.05 to 0.2 mol/dm$^3$. 
a $^{13}$C NMR spectrum of free MA at pH 3. The total Al concentration in the sample solutions ranged from 0.05 to 0.2 M. The peaks at 43.5 and 176.9 ppm can be assigned to the carbon atoms of the methylene and carboxyl groups in the free MA molecule, respectively. The other peaks are due to the DSS. In the $^{13}$C NMR spectra of the sample solutions in which the MA/Al molar ratio was 3 and 4, an additional two sets of signals (44.1, 44.3) and (177.4, 177.7) ppm were observed (Figs. 6(c) and 6(d)). The peak intensity ratio of free MA:combined MA was 7.0:8.5 (Fig. 6(d)). The calculation using the total MA concentration (0.2 M) in the sample solution indicated that the Al(\text{MA})_2^- and Al(\text{MA})_3^{3-} complexes can be formed. At the MA/Al of 4, the existing ratio of Al(\text{MA})_2^- and Al(\text{MA})_3^{3-} was approximately 1:4. In the $^{13}$C NMR spectra, the signals at (44.1, 177.4) and (44.3, 177.7) can be assigned to the carbon atoms in Al(\text{MA})_2^- and Al(\text{MA})_3^{3-}, respectively. On the other hand, in the sample solution in which the MA/Al molar ratio was 1 (Fig. 6(b)), a new peak at (43.8, 177.1) ppm was observed. Furthermore, because the peak due to the free MA was not observed, all of the MA interacted with Al$^{3+}$ under this condition. In addition, the peak due to the Al-MA complex can not be divided into two peaks, which indicates that all of the MA interacts with the Al$^{3+}$ as a bi-dentate ligand, and forms a chelate complex with a six-membered ring. The peak at (43.8, 177.1) can be assumed to correspond to the Al(\text{MA})_3^{3-} complex. From these results, three complex species (Al(\text{MA})_2^-, Al(\text{MA})_3^{3-} and Al(\text{MA})_4^+) are included in the peak at 2 ppm in the $^{27}$Al NMR spectrum at pH 3.

In OX and MA systems, we successfully proposed a new interpretation of the $^{27}$Al NMR spectra, that several complexes are contained in one peak. In a previous study, the peak assignment of the $^{27}$Al NMR spectra of Al$^{3+}$ and organic acid solution was conducted by the combination with potentiometry or an additively low for the chemical shift of the NMR spectrum. However, the lower symmetry of Al complex leads the extreme broadening by quadrupole interaction and the definite discussion of peak assignments is often more difficult than $^1$H and $^{13}$C NMR spectrum. Our result is useful information for investigating Al complexation with several organic compounds.

Interaction between aluminium ion and succinic acid

Figure 7 presents the $^{27}$Al and $^{13}$C NMR spectra of a mixed solution of Al$^{3+}$ and succinic acid (SU) with a SU/Al molar ratio of 5 at pH 3. In the $^{27}$Al NMR spectrum (Fig. 7(a)), only a slightly expanded peak was observed at 0 ppm. This indicates that the peak due to the Al-SU complex is included in the peak at around 0 ppm. This result is consistent with that of the Al$^{3+}$ and acetic acid mixed solution at pH 3. The $^{27}$Al NMR spectra of the Al$^{3+}$ and acetic acid mixed solution, which can act as a monodentate ligand, with various concentrations of acetic acid are summarized in Supporting Information (Fig. S-3). In the spectra of the acetic acid system, only the expanded peak at approximately 0 ppm was observed, which is similar to that of the spectra of the SU system. This peak can be assigned to the Al-acetic acid complex in which acetic acid combined as a monodentate ligand. Therefore, the expanded peak at approximately 0 ppm in the $^{27}$Al NMR spectra of the Al$^{3+}$ and SU mixed solutions can be assigned to the Al-SU complex with which SU combined as a monodentate complex. In $^{13}$C NMR spectrum (Fig. 7(b)), the peaks in 31.7 and 179.9 ppm can be assigned to the carbon atoms of the ethylene and carboxyl groups in free SU, respectively. In addition to these peaks, two other sets of signals (32.2, 34.1) and (180.7, 183.7) ppm were observed for the Al$^{3+}$ and SU mixed solution. Based on the structure of SU, it can form a chelate structure with a seven-membered ring if it binds as a bi-dentate ligand to Al$^{3+}$. However, the seven-membered ring is thermodynamically unstable. Therefore, SU is considered to interact with Al$^{3+}$ as a mono-dentate ligand, and the chemical structure of the Al-SU complex is Al(\text{SU})_2^+. With respect to the Al-SU complex, another complex, Al(\text{SU})_3^+, was also proposed. Kiss et al. also reported the additional peak at 4.6 ppm in $^{27}$Al NMR spectrum at pH 4.2, and assigned it to the Al(\text{SU})_3^+ with seven-membered ring. However, the additional peak at 4.6 ppm due to the Al(\text{SU})_3^+ could not be observed in our experimental condition. Therefore, only the Al(\text{SU})_2^+ complex existed in this study. In the $^{13}$C NMR spectrum (Fig. 7(b)), the peaks at 183.7 and 180.7 ppm may be assigned to the one carboxyl group combined with Al$^{3+}$, and another free carboxyl group that did not combine to Al$^{3+}$, respectively.

Conclusions

The complexation between Al$^{3+}$ and three dicarboxylic acids that strongly interact with Al$^{3+}$ was investigated at pH 3. In this study, we attempted to quantitatively investigate the chemical species of each Al-organic acid complex at pH 3 based on the total Al and the ligand concentrations, and on the quantitative.
In the succinic acid (SU) system, the obvious peak due to the Al-SU complex was not detected by 27Al NMR. However, in the oxalic acid (OX) system with various OX/Al molar ratios, we detected four Al-OX complexes (Al2(OX)4+, Al(OX)+, Al(OX)2– and Al(OX)33–) at pH 3. The 13C and 27Al NMR spectra of the Al16 and OX mixed solutions revealed that the peak at 16 ppm in 27Al NMR spectrum includes the complexes (Al(OX)2– and Al(OX)33–) at pH 3. Furthermore, in the malonic acid (MA) system, three complexes (Al(MA)+, Al(MA)2– and Al(MA)33–) are included in the peak at 2 ppm in the 27Al NMR spectrum at pH 3. We determined that the peaks in the 27Al NMR spectrum for an Al3+ and OX (or MA) mixed solution include some complexes, and that the ratio of these complexes depends on the ligand/Al molar ratio in the sample solution. In addition, new assignments of the 27Al NMR spectra of Al3+ and OX (or MA) mixed solution were proposed.

OX and MA can form a chelate structure because of the thermodynamic stability of the five- and six-membered rings. In the succinic acid (SU) system, the obvious peak due to the Al-Succinate complex was not detected by 27Al NMR. However, from the 13C NMR spectrum and a comparison of the 27Al NMR spectra for the Al-SU complex with that for the Al-acetate complex, it was suggested that the AlHSU2+ complex forms at pH 3. In the SU system, only a mono-dentate complex was detected, which suggests that the seven-membered ring structure is less stable.

Supporting Information

The peak area ratio of the deconvoluted 27Al NMR spectrum and the calculated Al concentration (Table S-1). Disagreements of the OX concentration between experimental OX concentration and calculated OX concentration based on the hypothesis that the peaks at 12 and 16 ppm in 27Al NMR spectra are due to the Al(OX)2– and Al(OX)33–, respectively (Table S-2). The calculated OX concentration based on the following two hypothesis (Table S-3). The composition of each complex in mixed solutions at various OX/Al molar ratio (Table S-4). The example of deconvoluted the 27Al NMR spectrum (Fig. S-1). The deconvoluted 27Al NMR spectrum of the mixed solution with an OX/Al molar ratio of 1.0 (Fig. S-2) and 27Al NMR spectra of Al and acetic acid mixed solution at pH 3 (Fig. S-3). This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

References