

ORIGINAL ARTICLE

Hikaru Aimi · Ko Tahara · Yasuji Kurimoto
Shigeru Yamauchi

Formation of a complex with aluminum by ozone-treated kraft lignins and their low molecular weight fragments

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Abstract The formation of a complex with aluminum by low molecular weight compounds and saponified ozone-treated kraft lignins was evaluated based on the decrease in pH of their solutions on the addition of 0.1 M AlCl_3 . Decreases in pH were observed with the solutions containing compounds having adjacent carboxyl groups (oxalic acid), carboxyl/alcoholic hydroxyl groups (glycolic acid), carboxyl/formyl groups (glyoxylic acid), and phenolic hydroxyl groups (protocatechuic acid) on the addition of 0.1 M AlCl_3 . The malonic and phthalic acids, having two carboxyl groups, were also effective. These results show that the compounds were effective in forming complexes with aluminum. This finding corresponds to the fact that aluminum toxicity is reduced by formation of a complex with aluminum, except in phthalic acid. The chemical structures stated above in ozone-treated kraft lignins contribute, at least partly, to the complex with aluminum. The pH of solutions containing saponified ozone-treated kraft lignins and alkaline-treated kraft lignin decreased more than that without modified kraft lignins on the addition of 0.1 M AlCl_3 , showing that they were effective in forming a complex with aluminum. The high molecular weight part of saponified ozone-treated kraft lignin was effective in forming a complex with aluminum and in reducing its toxicity.

Key words Aluminum · Complex · Lignin · Aluminum toxicity · Ozone

Introduction

Aluminum (Al) is the third most abundant element after silicon and oxygen and comprises about 7% of the earth's

crust. Most aluminum exists in an insoluble and harmless form to plants at neutral pH, but phytotoxic aluminum ions are eluted by the acidification of soil.¹ It is believed that aluminum toxicity is one of the main reasons for poor plant growth in acidic soils.^{1–8}

The toxicity of aluminum is reduced by the formation of a complex with some organic compounds. Bartlett and Riego⁹ reported that citric acid and EDTA (ethylenediaminetetraacetic acid) reduced aluminum toxicity in plant growth tests with maize. The complexes Al-citrate (1:1) and Al-oxalate (1:3) isolated from cell sap of hydrangea and buckwheat, respectively, did not cause symptoms of toxicity in plant growth tests with corn.^{10–13} Hue et al.¹⁴ reported that some acids could reduce aluminum toxicity, if they have two carboxyl groups, carboxyl/alcoholic hydroxyl groups, or carboxyl/phenolic hydroxyl groups positioned in a manner suitable for a five-membered or six-membered chelate structure with aluminum.

The relationship between complexation and reduction in toxicity of aluminum with modified lignins was first examined by Katsumata et al.^{15,16} Katsumata et al.^{15–18} and Wang et al.^{19,20} reported that lignins modified with alkaline oxygen or radical sulfonation reduced aluminum toxicity. Katsumata et al.¹⁶ observed the release of protons by potentiometric titration of solutions containing kraft lignins (KLs) modified with alkaline oxygen in the presence of aluminum. They attributed the release to the formation of a complex between the modified KL and aluminum. Complex of KL modified with alkaline oxygen and aluminum was also supported by the disappearance of the signal of aluminum ion after the addition of modified KL by ²⁷Al-NMR (nuclear magnetic resonance) analysis.¹⁶

The authors have reported that ozone-treated KLs reduced aluminum toxicity.^{21,22} Ozone treatment is advantageous for its rapid and highly efficient oxidation of lignin.²³ Ozone oxidized both phenolic- and non-phenolic-type structures in lignin.^{24,25} It is expected that the introduction of carboxyl groups into lignin by ozone oxidation could improve the ability of lignin to reduce aluminum toxicity, because the carboxyl group plays an important role in

H. Aimi (✉) · Y. Kurimoto · S. Yamauchi

¹Institute of Wood Technology, Akita Prefectural University,
11-1 Kaieisaka, Noshiro, Akita 016-0876, Japan
Tel. +81-185-52-6900; Fax +81-185-52-6924
e-mail: aimi-7980@iwt.akita-pu.ac.jp

K. Tahara

Department of Molecular and Cell Biology, Forestry and Forest
Products Research Institute, Ibaraki 305-8687, Japan

forming a complex with aluminum^{14,26–28} and reducing its toxicity.^{14,26}

In a previous paper,²⁹ plant growth tests were carried out with low molecular weight compounds to determine what types of functional groups contribute to reducing aluminum toxicity in saponified ozone-treated kraft lignins and their low molecular weight fragments. The low molecular weight compounds used contained carboxyl, formyl, methoxyl, alcoholic hydroxyl, and phenolic hydroxyl groups. These functional groups were expected to be present in ozone-treated KLs and their low molecular weight fragments. The compounds having two carboxyl groups (oxalic and malonic acids), adjacent carboxyl/alcoholic hydroxyl groups (glycolic acid), adjacent carboxyl/formyl groups (glyoxylic acid), and adjacent two phenolic hydroxyl groups (protocatechuic acid) reduced aluminum toxicity effectively.²⁹ These results indicate that the ability of ozone-treated KLs to reduce aluminum toxicity was partly the result of these chemical structures that they contained.

It is important to clarify why the ozone-treated KLs and compounds just stated reduced aluminum toxicity. The reduction is likely caused by the formation of a complex with aluminum, because complexation is known to play an important role in reducing aluminum toxicity. There is, however, no concrete evidence of complexes of ozone-treated KLs and aluminum.

In this study, complexes of ozone-treated KLs, low molecular weight compounds, and aluminum were examined by potentiometry. This technique has been commonly used for this purpose.^{26,30} The formation of complexes between aluminum and organic ligand leads to the release of protons from carboxyl and other acidic groups. It is known that the pH of a solution decreases on the addition of aluminum, if the solution contains a compound that forms a complex with aluminum.²⁶ In this study, complexation with aluminum was examined using a solution of initial pH 3.1 to avoid disturbance from acidity of the AlCl₃ solution and that of initial pH 4.8, the same pH as used in plant growth tests.^{21,22,29}

Materials and methods

Materials

The compounds used are shown in Fig. 1. EDTA (ethylenediamine-*N,N,N',N'*-tetraacetic acid, disodium salt, dehydrate) (1), citric acid (2), acetic acid (3), oxalic acid (4), malonic acid (5), phthalic acid (6), glycolic acid (7), glycerol (8), glyoxylic acid monohydrate (9), DL-glyceraldehyde (10), and protocatechuic acid (12) were purchased from Wako (Osaka, Japan). Vanillic acid (13) and *p*-hydroxybenzoic acid (11) were obtained from Tokyo Chemical Industry (Tokyo, Japan). All these compounds were used without further purification.

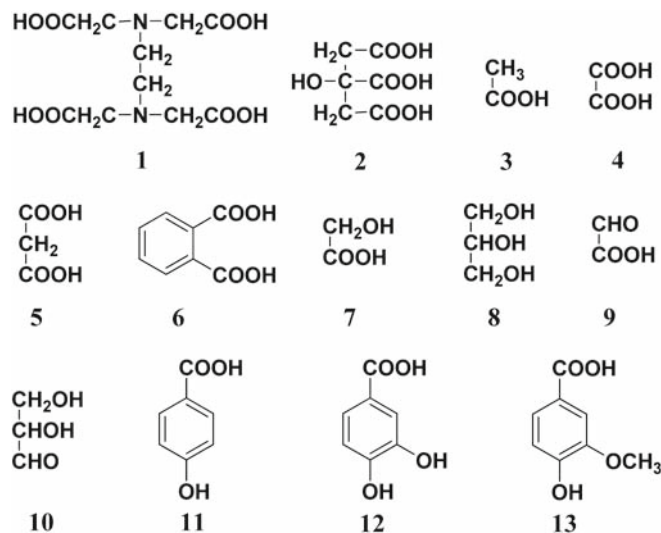


Fig. 1. Chemical structure of each compound used. 1, EDTA (ethylenediaminetetraacetic acid); 2, citric acid; 3, acetic acid; 4, oxalic acid; 5, malonic acid; 6, phthalic acid; 7, glycolic acid; 8, glycerol; 9, glyoxylic acid; 10, glyceraldehyde; 11, *p*-hydroxybenzoic acid; 12, protocatechuic acid; 13, vanillic acid

Modification of KL with ozone followed by alkali^{21,22}

Crude softwood KL from the Shimada Mill of Tokai Pulp and Paper (Shizuoka, Japan) was purified by the aqueous dioxane (95%, v/v)/diethyl ether method.²¹ The yield of purified KL was 72%.²¹

The purified KL (1 g) was suspended in 60 ml of a solvent mixture consisting of acetic acid/water/methanol (16:3:1, v/v/v) and oxidized by bubbling with oxygen containing ozone (ca. 3.7%, w/w) at a rate of 0.5 l min⁻¹ using an ED-OG-R3Lt ozone generator (Ecodesign, Saitama, Japan) for 0.5, 1.0, 2.0, and 4.0 h at 0°C with stirring. The reaction mixture was bubbled with oxygen for a further 10 min and then evaporated dry. The KL modified with ozone for 4 h was suspended in water and freeze-dried. The residues were dried under reduced pressure at 45°C overnight. The yields of all ozone-treated KLs were 97%–111%.²¹ The KLs modified with ozone for 0.5, 1.0, 2.0, and 4.0 h are referred to as 0.5-O₃, 1.0-O₃, 2.0-O₃, and 4.0-O₃, respectively.

The purified KL or ozone-treated KL (1 g each) was dissolved in 0.1 M NaOH (250 ml) and kept at room temperature overnight. The solution was applied to a cation-exchange column (H⁺ form, 2.0 Eq l⁻¹; Amberlite IR120B, Organo, Tokyo, Japan). The eluates were neutralized with diluted NaOH, concentrated in vacuo and freeze-dried. The yields of the products were 104%–122% based on the starting materials. The products from KL, 0.5-O₃, 1.0-O₃, 2.0-O₃, and 4.0-O₃ are referred to as KLA, 0.5-O₃A, 1.0-O₃A, 2.0-O₃A, and 4.0-O₃A, respectively.

Dialysis of saponified 0.5-O₃

The ozone-treated KL 0.5-O₃ (700 mg) was dissolved in 0.1 M NaOH (175 ml) and kept at room temperature overnight. The solution was then neutralized with diluted HCl

and subjected to dialysis against distilled water for 3 days with a size 27 dialysis membrane [molecular weight cutoff (MWCO), 12,000–14,000; Wako, Osaka, Japan]. The dialysate was evaporated and freeze-dried. The yield of product was 80% for 0.5-O₃, and the product was named 0.5-O₃AD.

Analysis of complexation by potentiometry

Known amounts of low molecular weight compounds and modified KLs were dissolved in water (200 ml), and the solutions were adjusted to a pH of 3.0 or 4.6–4.8 with diluted HCl and/or NaOH and a volume of 250 ml with water. The final concentration of solutions containing low molecular weight compounds was 2 mM and that of those containing modified KLs was 360 mg l⁻¹. The final pH of the solutions adjusted to pH 3.0 during the preparation was 3.1–3.3. The pH of solutions adjusted to pH 4.6–4.8 during the preparation was readjusted to 4.8 with diluted HCl and/or NaOH.

Determination of complexes formed with aluminum was carried out by the addition of 0.1 M AlCl₃ (100 μl) to 50 ml solution containing low molecular weight compounds or modified KLs at room temperature with stirring. Changes in pH of solutions were measured after every addition of 100 μl 0.1 M AlCl₃. Measurements were made in duplicate for each compound and modified KL. No precipitate was observed after the addition of aluminum when the solution containing low molecular weight compounds were used, while precipitates were observed with the use of the solution containing modified KLs after the addition of 100–400 μl 0.1 M AlCl₃, except for 4.0-O₃A. A blank test was conducted without the compound or modified KL. The experiment was carried out under ambient conditions because the use of a blank solution or solution containing oxalic acid (4) did not make any difference with or without nitrogen bubbling.

Analysis of soluble Al in solutions containing modified KLs and aluminum by the inductively coupled plasma (ICP) method

KLA or ozone-treated KL (36 mg each) was dissolved in 100 ml water, and the solution was adjusted to pH 4.8 with diluted HCl. After 0.1 M AlCl₃ (1 ml) was added, the solution was kept at room temperature for 1 h with stirring and then overnight without stirring. Precipitates were removed with a membrane filter (mixed cellulose ester, pore size 0.45 μm; Adventec, Tokyo, Japan) and washed twice with water adjusted to pH 4.0 to avoid the precipitation of aluminum. The filtrate and washings were combined and the total volume was adjusted to 250 ml with water (pH 4.0). The Al concentration in the solution was determined with an inductively coupled plasma optical emission spectrometer (Optima 4300DV; Perkin Elmer, Waltham, MA, USA). Ultraviolet (UV) absorption of the solution was measured with a UV-3100PC (Shimadzu, Kyoto, Japan). A blank test was carried out without modified KL.

Plant growth test

The plant growth test was reported in detail previously.²¹

Seeds of radish (*Raphanus sativus* L. var. *radicula* Pers.) were sterilized in a 70% ethanol aqueous solution for 30 s and then in a 1% sodium hypochlorite solution for 10 min. Ten young seedlings were transplanted to a nylon mesh holder that was floating on 700 ml of the hydroponic solution. A solution with 1.2 mM CaCl₂ at pH 4.8 was used as a nutrient solution. The plant growth test was carried out in the presence and absence of AlCl₃ (37.5 μM) and 0.5-O₃AD (10–250 mg l⁻¹) at 23.5°C with continuous air bubbling. The pH of hydroponic solutions was adjusted to 4.8 with diluted HCl and/or NaOH two times a day during the plant growth test. Primary root lengths were measured with a ruler after a 3-day growth period. Abnormal values were removed using Dixon's Q method (*n* = 10).³¹ The results were evaluated statistically by the *t* test method for the assessment of significance.³¹

Results and discussion

Determination of complexation between low molecular weight compounds and aluminum

The formation of a complex between aluminum and an organic ligand leads to the release of protons from carboxyl and other acidic groups.²⁶ Complexation involving aluminum was examined based on this phenomenon. First, a solution with an initial pH of 3.1 was used because the 0.1 M AlCl₃ solution was weakly acidic (pH 3.1). A blank test with water of initial pH 3.1 showed that the pH of the solution was almost constant with the addition of 0.1 M AlCl₃ (Fig. 2a).

EDTA (1) is a chelating agent, and citric acid (2) was reported to form a complex with aluminum.¹⁰ Solutions containing EDTA and citric acid (1, 2 in Fig. 1) were tested to determine whether the pH decreased effectively on addition of the 0.1 M AlCl₃ solution. The changes in pH are shown in Fig. 2a. The pH of the solutions decreased more with the compounds than without compounds (blank) on the addition of Al.

The formation of a complex with aluminum was examined with the low molecular weight compounds 3–13 (Fig. 1), containing carboxyl, formyl, methoxyl, alcoholic hydroxyl, and phenolic hydroxyl groups. These functional groups were expected to be present in ozone-treated KLs and their low molecular weight fragments.

A five-membered or six-membered ring is the most stable structure for chelating metals,³⁰ and carboxyl groups play an important role in the formation of a complex with aluminum.^{14,26–28} Acetic, oxalic, malonic, and phthalic acids (3–6 in Fig. 1) were examined to confirm the importance of the relative positions of the carboxyl groups. Figure 3a shows the changes in pH of solutions containing these compounds with the addition of Al. The pH of the solution containing acetic acid (3) was almost constant, showing that a compound having only one carboxyl group did not affect

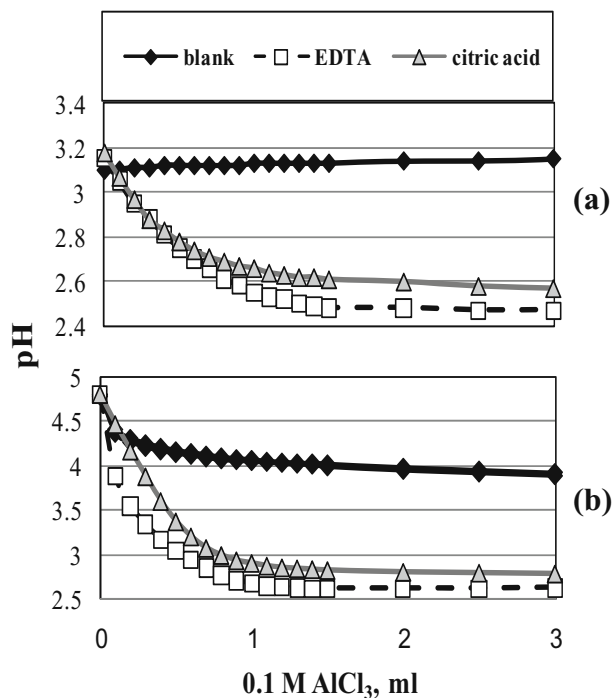


Fig. 2. Changes in pH of the solutions containing EDTA (1) (—□—) and citric acid (2) (—△—) on the addition of 0.1 M AlCl_3 . **a** Solutions with an initial pH of 3.1; **b** solutions with an initial pH of 4.8. Blank (—◆—), solutions without compounds

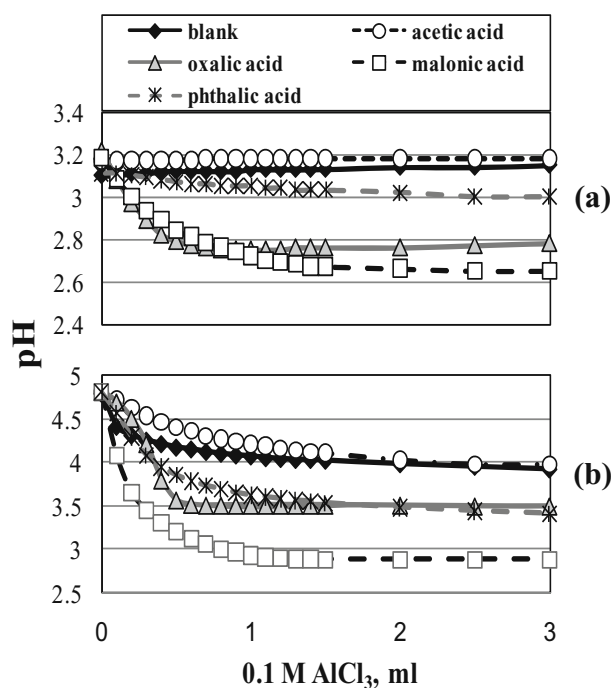


Fig. 3. Changes in pH of the solutions containing acetic acid (3) (—○—), oxalic acid (4) (—△—), malonic acid (5) (—□—), and phthalic acid (6) (—*—) on the addition of 0.1 M AlCl_3 . **a** Solutions with an initial pH of 3.1; **b** solutions with an initial pH of 4.8. Blank (—◆—), solutions without compounds

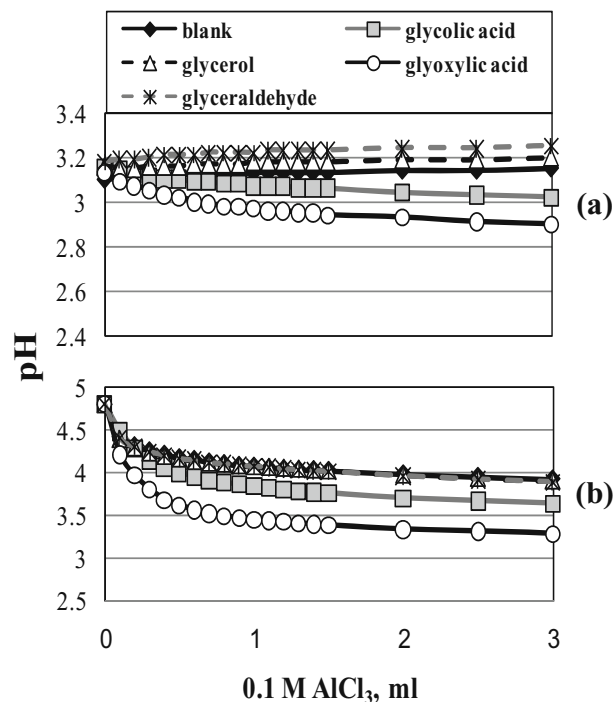


Fig. 4. Changes in pH of the solutions containing glycolic acid (7) (—□—), glycerol (8) (—△—), glyoxylic acid (9) (—○—), and glyceraldehyde (10) (—*—) on the addition of 0.1 M AlCl_3 . **a** Solutions with an initial pH of 3.1; **b** solutions with an initial pH of 4.8. Blank (—◆—), solutions without compounds

pH on the addition of Al. The pH of the solutions with oxalic acid (4) and malonic acid (5) clearly decreased with the addition of Al. Oxalic acid (4) and malonic acid (5) have two carboxyl groups suitably positioned to make a five-membered or six-membered ring with aluminum. The complex of Al-oxalate (1:3) was isolated from cell sap of buckwheat.^{11,12} The pH of the solution containing phthalic acid (6) also decreased, although only slightly.

Complexation with aluminum predominantly involved functional groups containing oxygen.^{3,26} Experiments were carried out with glycolic acid, glycerol, glyoxylic acid, and glyceraldehyde (7–10 in Fig. 1) to examine the contributions of carbonyl and alcoholic hydroxyl groups. The results are shown in Fig. 4a. The pH of solutions containing glycolic acid (7) and glyoxylic acid (9) decreased on the addition of Al, showing that the adjacent carboxyl/alcoholic hydroxyl groups and carboxyl/formyl groups contributed to the complex with aluminum. The magnitudes of the decrease in pH with glycolic acid (7) and glyoxylic acid (9) were small, which may be attributable to the fact that glycolic acid (7) and glyoxylic acid (9) have one carboxyl group. The pH of the solution containing glycerol (8), having two adjacent alcoholic hydroxyl groups, did not decrease with the addition of Al. This observation confirms the findings of Schnitzer et al.²⁷ that alcoholic hydroxyl groups did not participate in forming a complex with aluminum. The pH of the solution containing glyceraldehyde (10), having adjacent formyl/alcoholic hydroxyl groups, also did not change.

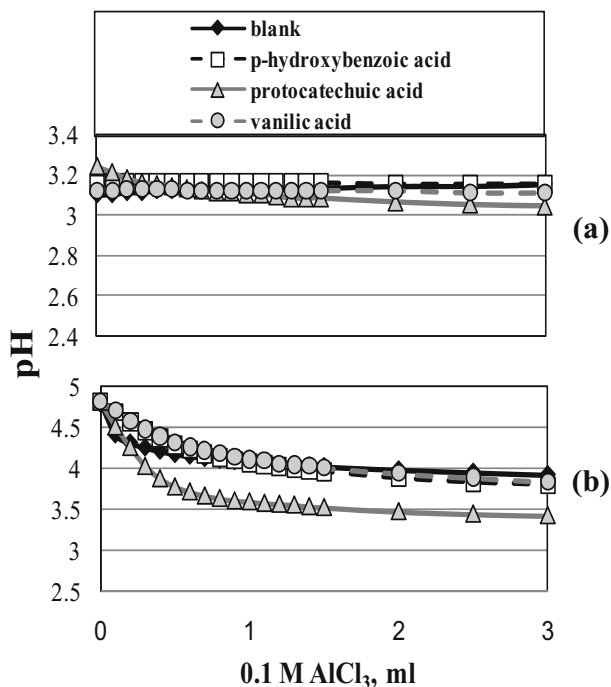


Fig. 5. Changes in pH of the solutions containing *p*-hydroxybenzoic acid (11) (—□—), protocatechuic acid (12) (—△—), and vanillic acid (13) (—○—) on the addition of 0.1 M AlCl₃. **a** Solutions with an initial pH of 3.1; **b** solutions with an initial pH of 4.8. Blank (—◆—), solutions without compounds.

Experiments were also conducted with solutions containing *p*-hydroxybenzoic, protocatechuic, and vanillic acids (11–13 in Fig. 1) to examine the contributions of the phenolic hydroxyl group and its methyl ether (methoxyl group). Only the pH of the solution containing protocatechuic acid (12) decreased (Fig. 5a), although the magnitude of change was very small. One phenolic hydroxyl group or guaiacyl nuclei did not affect the pH.

Relationship between the formation of a complex and reduction in the toxicity of aluminum

It was suggested by Vance et al.²⁶ and Barceló and Poschenrieder³² that the contribution of phenolic hydroxyl groups to reducing aluminum toxicity was less efficient in acidic solutions. Ofei-Manu et al.³³ reported that the aluminum-binding capacity of compounds having the catechol structure was greatly lower at pH 4.5 than at pH 7.0. These results suggest that the ability of a ligand to form a complex with aluminum depends on the pH of the solution.

Next, the effect of complexation was reexamined with compounds 3–13 (see Fig. 1) at pH 4.8, the same pH as used previously in plant growth tests.²⁹

The results for solutions containing EDTA and citric acid (1, 2 in Fig. 1) are shown in Fig. 2b. A decrease in pH was observed even with the blank on the addition of 0.1 M AlCl₃ because of the acidity of the 0.1 M AlCl₃ solution itself. However, the pH of solutions containing EDTA (1) and citric acid (2) decreased more than that of the blank.

This result clearly shows that a complex formed even with the solution of initial pH 4.8.

Figures 3b, 4b, and 5b show the changes in pH of solutions containing compounds 3–13 (Fig. 1) on the addition of Al. Oxalic acid (4), malonic acid (5), phthalic acid (6), glycolic acid (7), glyoxylic acid (9), and protocatechuic acid (12) lowered the pH compared to the blank. It has been reported that oxalic acid (4), malonic acid (5), glycolic acid (7), glyoxylic acid (9), and protocatechuic acid (12) reduced aluminum toxicity.²⁹ These findings demonstrate that the compounds that could form a complex with aluminum were effective in reducing its toxicity, except for phthalic acid (6). It is not clear why phthalic acid (6) was effective in forming a complex with aluminum but had no effect on toxicity. Inhibition of root elongation, a typical symptom of aluminum toxicity,³⁴ was not observed in the plant growth test with phthalic acid (6) in the absence of aluminum,²⁹ showing that phthalic acid (6) itself had no toxic effect. It was reported that a seven-membered ring, such as that found in the aluminum complex with two carboxyl groups in phthalic acid (6) or succinic acid, has weak stability because of steric hindrance.²⁶

The compounds that could decrease the pH of the solution with an initial pH of 4.8 more than the blank on the addition of Al were the same as those examined with an initial pH of 3.1 (Figs. 3a, 4a, and 5a). However, the pH of solutions containing phthalic acid (6) and protocatechuic acid (12) decreased more effectively when the initial pH was 4.8 rather than 3.1 (Figs. 3, 5). These results show that the pH of the solution affects the ability of these compounds to form a complex with aluminum.

Complexes of saponified ozone-treated KLS and alkaline-treated KL with aluminum

It has been reported that saponified ozone-treated KLS (0.5-O₃A, 1.0-O₃A, 2.0-O₃A, and 4.0-O₃A) and alkaline-treated KL (KLA) reduced aluminum toxicity effectively.²² It is, however, not known if these modified KLS form a complex with aluminum or not. The modified KLS (KLA, 0.5-O₃A, 1.0-O₃A, 2.0-O₃A, and 4.0-O₃A) were subjected to experiments to examine the relationship between reduction in aluminum toxicity and complexation with these modified KLS. Figure 6 shows the changes in pH of the solutions containing KLA, 0.5-O₃A, 1.0-O₃A, 2.0-O₃A, and 4.0-O₃A with the addition of Al solution. The pH of the solutions containing these modified KLS decreased more than that of the blank. These results show that saponified ozone-treated KLS and alkaline-treated KL were effective in forming a complex with aluminum.

Some organic ligands form precipitates after forming a complex with metals.³⁰ In this study, precipitates formed after the addition of aluminum to the solutions containing modified KLS, except for 4.0-O₃A. The concentration of soluble aluminum is expected to decrease after the formation of precipitates, if the complex contains modified KLS and aluminum. The precipitates that formed after the addition of aluminum were removed, and the concentration of

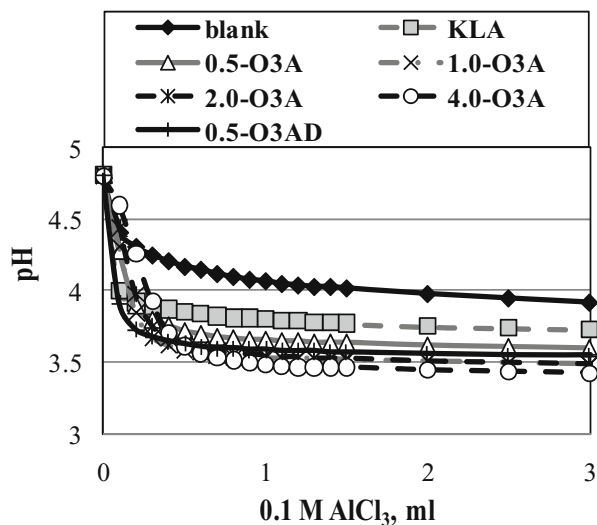


Fig. 6. Changes in pH of the solutions containing KLA (—□—), 0.5-O₃A (—△—), 1.0-O₃A (—×—), 2.0-O₃A (—*—), 4.0-O₃A (—○—), and 0.5-O₃AD (—|—) on the addition of 0.1 M AlCl₃. Solutions with an initial pH of 4.8 were used. Blank (—◆—), a solution without modified kraft lignin (KL). KLA, alkaline-treated KL; 0.5-O₃A, KL modified with ozone for 0.5 h and alkali; 1.0-O₃A, KL modified with ozone for 1.0 h and alkali; 2.0-O₃A, KL modified with ozone for 2.0 h and alkali; 4.0-O₃A, KL modified with ozone for 4.0 h and alkali; 0.5-O₃AD, high molecular weight dialysate of 0.5-O₃A

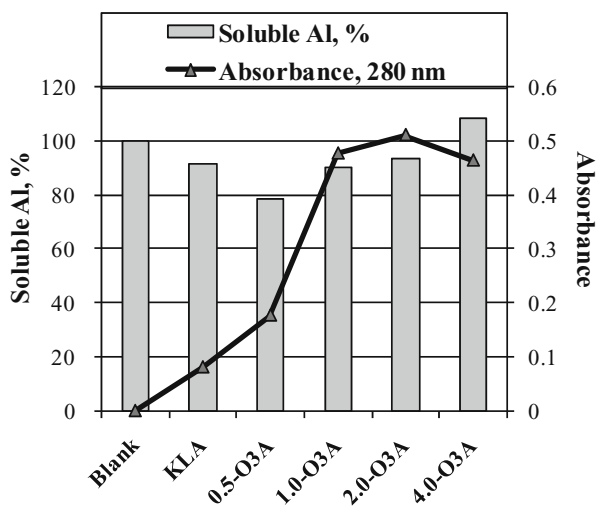


Fig. 7. Soluble Al (gray bars) and UV absorbance (280 nm, —△—) of the solutions containing modified KLA and Al after removing precipitates. Soluble Al was based on the solution containing Al (blank, 9.8 mg l⁻¹)

soluble Al was determined by ICP analysis. The results are shown in Fig. 7. The concentration of soluble Al decreased more with the modified KLA, except for 4.0-O₃A, than with the blank, suggesting that these modified KLA form a complex with aluminum. Soluble Al in the solution containing 1.0-O₃A, 2.0-O₃A, and 4.0-O₃A increased more than in that containing 0.5-O₃A, possibly the result of the formation of soluble complexes because protons were released after the addition of aluminum (see Fig. 6). The increase in UV absorbance at 280 nm for 1.0-O₃A, 2.0-O₃A, and 4.0-O₃A

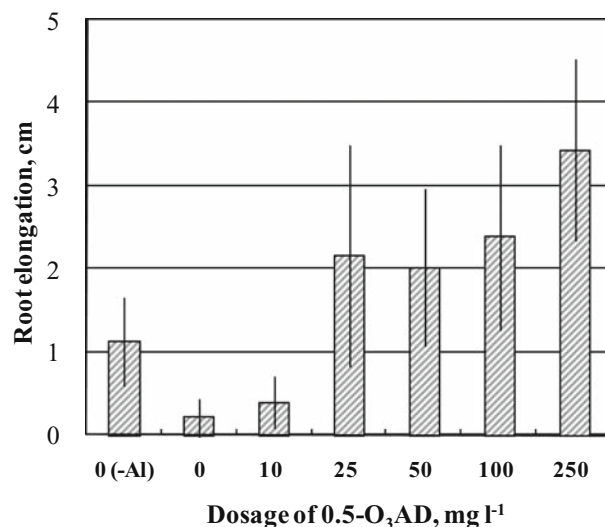


Fig. 8. Effect of 0.5-O₃AD on root elongation in radish. Hydroponic solution, containing 1.2 mM CaCl₂ and 37.5 μM AlCl₃ at pH 4.8; 0(-Al), hydroponic solution without aluminum. Growth period was 3 days (*n* = 10). Error bars represent ± SD

supports the idea of the formation of a soluble complex between modified KLA and aluminum (see Fig. 7). The formation of a soluble complex between ozone-treated KLA and aluminum may be attributable to the introduction of carboxyl groups in modified KLA by ozone treatment. The concentration of soluble Al in the solution containing Al and 4.0-O₃A (10.6 mg l⁻¹) was higher than that in the blank (9.8 mg l⁻¹); this can be explained by the contribution of aluminum originally contained in 4.0-O₃A (0.6 mg l⁻¹).

The low molecular weight part of saponified ozone-treated KL is believed partly effective in forming a complex with aluminum, because low molecular weight compounds that reduced aluminum toxicity were found in ozone-treated KLA.^{21,22,29} It was reported that the amount of oxalic acid (4) increased in KL with ozone treatment.^{21,22} Malonic acid (5), glycolic acid (7), and glyoxylic acid (9) were detected as minor reaction products in trimethylsilylated ozone-treated KLA.^{21,22,35} These compounds were confirmed to form a complex with aluminum in the present study. It is, however, not clear if the high molecular weight part of saponified ozone-treated KL is also effective in forming a complex with aluminum and reducing its toxicity. The high molecular weight part of saponified 0.5-O₃ was prepared by dialysis (0.5-O₃AD) and subjected to a plant growth test and complexation with aluminum. The effect of 0.5-O₃AD on root elongation in radish is shown in Fig. 8. If a reduction in aluminum toxicity is defined as a recovery of root elongation to the level without aluminum [0(-Al)], this was attained with 0.5-O₃AD. As shown in Fig. 6, the pH of the solution containing 0.5-O₃AD decreased with the addition of Al solution. This finding clearly shows that the high molecular weight part of saponified ozone-treated KL was also effective not only in reducing aluminum toxicity but also in forming a complex with aluminum.

Ozone-treated KLA were effective in the recovery of root elongation of radish in the presence of aluminum.^{21,22} The

recovery with ozone-treated KLs was assumed to result from the ability to reduce aluminum toxicity and/or accelerate root elongation. It is, however, not known which, or if both, of these factors contributed to the recovery. The results obtained in this study show that the ability of ozone-treated KLs to reduce aluminum toxicity contributed, at least partly, to the recovery of root elongation in radish, because complexation is key to the reduction in toxicity. Katsumata et al.^{15,18} reported that the adsorption of aluminum to root tips was effectively prohibited by the presence of KLs modified with alkaline oxygen. This result also supports the idea that the recovery of root elongation with oxidized KLs cannot be explained only by the ability to accelerate root elongation.

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References

- Matsumoto H (2000) Cell biology of aluminum toxicity and tolerance in higher plants. *Int Rev Cytol* 200:1–46
- Aimi R, Murakami T (1964) Cell-physiological studies on the effect of aluminum on the growth of crop plants (in Japanese). *Bull Nat Inst Agric Sci D11:331–396*
- Ma JF (2000) Role of organic acids in detoxification of aluminum in higher plants. *Plant Cell Physiol* 41:383–390
- Ma JF, Ryan PR, Delhaize E (2001) Aluminum tolerance in plants and the complexing role of organic acids. *Trends Plant Sci* 6: 273–278
- Ma JF, Furukawa J (2003) Recent progress in the research of external Al detoxification in higher plant: a minireview. *J Inorg Biochem* 97:46–51
- Kochian LV (1995) Cellular mechanisms of aluminum toxicity and resistance in plants. *Annu Rev Plant Physiol Plant Mol Biol* 46: 237–260
- Kochian LV, Hoekenga OA, Piñeros MA (2004) How do crop plants tolerate acid soil? Mechanisms of aluminum tolerance and phosphorous efficiency. *Annu Rev Plant Biol* 55:459–493
- Foy CD (1984) Physiological effects of hydrogen, aluminum, and manganese toxicities in acid soil In: Adams F (ed) *Soil acidity and liming*. Agronomy Monograph no. 12, 2nd edn. ASA-CSSA-SSSA, Madison, WI, pp 57–97
- Bartlett RJ, Riego DC (1972) Effect of chelation on the toxicity of aluminum. *Plant Soil* 37:419–423
- Ma JF, Hiradate S, Nomoto K, Iwashita T, Matsumoto H (1997) Internal detoxification mechanism of Al in hydrangea. *Plant Physiol* 113:1033–1039
- Ma JF, Zheng SJ, Matsumoto H (1997) Detoxifying aluminium with buckwheat. *Nature (Lond)* 390:569–570
- Zheng SJ, Ma JF, Matsumoto H (1998) High aluminum resistance in buckwheat. I. Al-induced specific secretion of oxalic acid from root tip. *Plant Physiol* 117:745–751
- Shen R, Ma JF, Kyo M, Iwashita T (2002) Compartmentation of aluminum in leaves of an Al-accumulator, *Fagopyrum esculentum* Moench. *Planta (Berl)* 215:394–398
- Hue NV, Graddock GR, Adams F (1986) Effect of organic acids on aluminum toxicity in subsoils. *Soil Sci Soc Am J* 50:28–34
- Katsumata K, Meshitsuka G (2002) Modified kraft lignin and its use for soil preservation In: Hu TQ (ed) *Chemical modification, properties and usage of lignin*. Kluwer, New York, pp 151–165
- Katsumata SK, Shintani H, Meshitsuka G (2003) Mechanism of detoxification of aluminum ions by kraft lignin treated with alkaline oxygen. *J Wood Sci* 49:93–99
- Saito K, Nakanishi MT, Matsubayashi M, Meshitsuka G (1997) Development of new lignin derivatives as soil conditioning agents by radical sulfonation and alkaline-oxygen treatment. *Mokuzai Gakkaishi* 43:669–677
- Katsumata SK, Maruyama M, Meshitsuka G (2001) Reduction of aluminum toxicity to radish by alkaline oxygen treated kraft lignin. *J Wood Sci* 47:129–134
- Wang D, Katsumata SK, Meshitsuka G (2005) Characterization of lignin fragments in alkaline oxygen-stage waste liquor as soil-conditioning agent. *J Wood Sci* 51:357–362
- Wang D, Katsumata SK, Meshitsuka G (2005) Effect of low molecular weight lignin fragments including oxalic acid in alkaline-oxygen stage waste liquor on Al toxicity. *J Wood Sci* 51:634–639
- Aimi H, Ohmura S, Kato T, Nakahara T, Shimizu K (2008) Development of acid soil conditioning agent from lignin by ozone treatment I. *J Wood Sci* 54:214–219
- Aimi H, Ohmura S, Uetake M, Shimizu K (2009) Development of acid soil conditioning agent from lignin by ozone treatment II. *J Wood Sci* 55:121–125
- Sarkanen KV, Islam A, Anderson CD (1992) Ozonation. In: Lin SY, Dence CW (eds) *Methods in lignin chemistry*. Springer-Verlag, Berlin, pp 387–406
- Kaneko H, Hosoya S, Iiyama K, Nakano J (1983) Degradation of lignin with ozone. Reactivity of lignin model compounds toward ozone. *J Wood Chem Technol* 3:399–411
- Eriksson T, Gierer J (1985) Studies on the ozonation of structural elements in residual kraft lignins. *J Wood Chem Technol* 5:53–84
- Vance GF, Stevenson FJ, Sikora FJ (1996) Environmental chemistry of aluminum-organic complexes. In: Sposito G (ed) *The environmental chemistry of aluminum*, 2nd edn. Lewis, Boca Raton, pp 169–220
- Schnitzer M, Skinner SIM (1965) Organo-metallic interactions in soils: 4. Carboxyl and hydroxyl groups in organic matter and metal retention. *Soil Sci* 99:278–284
- Tam SC, McColl JG (1990) Aluminum- and calcium-binding affinities of some organic ligands in acidic conditions. *J Environ Qual* 19:514–520
- Aimi H, Uetake M, Shimizu K (2009) Effective combinations of functional groups in chemically modified kraft lignins for reduction of aluminum toxicity. *J Wood Sci* 55:220–224
- Martell AE, Calvin M (1952) *Chemistry of the metal chelate compounds*. Prentice-Hall, Englewood Cliffs, NJ
- Miller JN, Miller JC (1988) *Statistics and chemometrics for analytical chemistry*, 2nd edn. Ellis Horwood, Chichester, UK
- Barceló J, Poschenrieder C (2002) Fast root growth responses, root exudates, and internal detoxification as clues to the mechanisms of aluminum toxicity and resistance: a review. *Environ Exp Bot* 48: 75–92
- Ofei-Manu P, Wagatsuma T, Ishikawa S, Tawarayama K (2001) The plasma membrane strength of the root-tip cells and root phenolic compounds are correlated with Al tolerance in several common woody plants. *Soil Sci Plant Nutr* 47:359–375
- Tahara K, Yamanoshita T, Norisada M, Hasegawa I, Kashima H, Sasaki S, Kojima K (2008) Aluminum distribution and reactive oxygen species accumulation in root tips of two *Melaleuca* trees differing in aluminum resistance. *Plant Soil* 307:167–178
- Tsutsumi Y, Islam A, Anderson CD, Sarkanen KV (1990) Acidic permanganate oxidations of lignin and model compounds: comparison with ozonolysis. *Holzforschung* 44:59–66