Effects of pH, Iron and Metal Concentration on Aluminium Coordination

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We have investigated the likelihood of aluminium (Al) being excreted in based on its binding capacity with dietary fiber (DF). The results of our previous study revealed that DF extracted from highly viscous food items such as aloe, okra, moroheiya, and wakame has strong metal binding capacity. Therefore, in the present study, the effects of pH, the presence of iron (Fe [II, III]) and metal (Al and Fe) concentrations on the binding capacity of Al to DF were examined. In both insoluble and soluble fractions extracted from these food items, the amount of Fe binding was higher than that of Al, except for the IDF fraction from hijiki. Furthermore, it was confirmed that, in the presence of both Al and Fe, Fe had higher affinity to alginic acid in gel filtration. For a decrease in pH from 3.5 to 2.0, the levels of both Al and Fe binding to alginic acid were reduced, with a greater degree of reduction observed for Al. For incremental increases in the concentrations of Al and Fe together, the level of Al binding to alginic acid was slightly increased only at pH 2.7 ± 0.3.

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Keywords: aluminium (Al), iron (Fe), pH, dietary fiber (DF), competitive binding.

INTRODUCTION

In June 2002, the Japanese Ministry of Health, Labor, and Welfare extended the limitation on the use of drugs containing aluminium (Al), which had been applied only to persons with renal diseases, to include healthy persons and called attention to long-term continuous usage.

Currently, the WHO sets the limit of Al intake to less than 350 mg per week. However, it is highly likely that intake exceeds 1,000 mg per day due to the ingestion of food items and additives with high levels of Al and also drugs containing Al-based drugs. Long-term ingestion of high levels of Al results in increased accumulation of Al in bone (Talwar et al. 1986; Nicar et al. 1992; Capdevielle et al. 1998; Teraki et al. 1998), organs, and the brain (Ecelberger et al. 1994; Struys-Ponsar et al. 1997; Nayak 2002; Kametani et al. 2003), and heightens the risks of osteomalacia, cardiac diseases, and several nervous system diseases, such as Parkinson’s disease, amyotrophic lateral sclerosis, and Alzheimer’s disease (Yokei 2000; Kawahara 2001). Since Al is widely distributed, ingestion of Al to some degree cannot be avoided. Furthermore, renal function depends on the health status of individuals and is lowered by aging (Moore et al. 2000); thus, it is assumed that the renal function of some healthy persons may be as low as that of patients with renal diseases. Therefore, it is desirable that Al ingested in daily life is excreted without being absorbed.

We have been investigating the possibility of Al excretion in terms of Al binding capacity to dietary fiber (DF), and clarified that significant effects of Al excretion can be expected for some types of DF (Takeyama et al. 1996, 2002; Fukushima et al. 2005). However, Al is not present alone, Al are coexist with Fe in most foods. So we investigated the effect of pH on the competitive binding of Al and iron (Fe) to DF. In the present study, soluble DF and insoluble DF were fractionated from DF and used for experiments. Additionally, since metals co-exist in ingested DF, we also investigated the effects of Fe, and metal concentrations on the binding of Al to alginic acid, which has particularly superior metal binding capacity.

MATERIALS AND METHODS

Materials

Commercially available okra, moroheiya (nalta jute: Corchorus olitorius L.), celery (Apium graveolens var. dulce), eryngii (Pleurotus eryngii), hijiki (edible brown algae: Hiyikia fusiforme), and wakame (Undaria

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*pinratifida*) were purchased. Aloe was purchased from the Aloe Vera Association in Japan. DF was extracted from these foods using Prosky's enzymatic-gravimetric method (Prosky et al. 1988) using tannamyl, protease, and amyloglucosidase. Extracted fiber was divided into 2 fractions: IDF, insoluble dietary fiber and SDF, soluble dietary fiber. Each fraction was subjected to 5- to 7-day dialysis in pure water (the dialysate of approximately 20 times the volume of each DF suspension was exchanged twice daily), and then freeze-dried until analysis.

Sodium alginate and alginic acid were purchased from Wako Pure Chemical Industries, Ltd. (Japan).

**Methods**

1. Measurements of the levels of Al and Fe binding to IDF and SDF fractions

   The same method was adopted for the measurement of Al and Fe binding for the IDF and SDF fractions. Samples (20 mg) of each DF fraction from each food sample were suspended in 5 ml of ultrapure water, to which 1 ml of each 40 ppm of Al and Fe standard solutions was added, and pH was adjusted to 3.5-2.0 with 0.01 mol/l nitric acid.

   After adjusting the total volume to 10 ml, the solution was incubated at 37°C for 1 h with shaking to facilitate the binding of Al and Fe ions. After centrifuging the solution at 20,000×g for 15 min, 2 ml of supernatant was aliquoted to a quartz test tube, and 0.5 ml of concentrated nitric acid was added for decomposition and desiccation used for block heater at 100°C. The pellet remaining in the quartz tube was resuspended in ultrapure water, 8 ml of 0.3 mol/l nitric acid was added, and then the total volume was adjusted to 100 ml with ultrapure water. The amount of Al and Fe in this solution was analyzed on a HITACHI Z-9000 atomic absorption spectrophotometer using a tube cuvette. Measurement was carried out with drying at 80-120°C for 30 s, ashing at 630°C for 30 s, and atomization at 3,000°C for 10 s. The sample size used for each analysis was 20 μl, and hollow cathode lamps for Al (wavelength 309.3 nm) and Fe (wavelength 248.3 nm) were utilized in the analysis, and the sample quantity was calculated using standard curves. The level of metal binding was calculated by subtracting the quantity of metal in the supernatant after centrifugation from that of the metal first added. The value calculated from dietary fiber sample without any addition of metals (a blank value) was subtracted from the calculated values.

   Aluminium and iron standard solutions for atomic absorption spectrometry, Al or Fe (1,000 mg/ml) · HNO₃ (0.2 mol/l) sol were purchased from KANTO Chemical co., Inc. (Japan). Appropriate dilutions were performed in ultrapure water.

2. Effect of Al and Fe concentration on the competitive binding capacity of Al to alginic acid

   Aliquots (10 mg) of alginic acid were placed in test tubes and resuspended in 4 ml of ultrapure water with stirring, to which 1 ml of each 40, 80, and 160 ppm of Al or Fe solutions was added. The total volume was adjusted to 10 ml with ultrapure water to produce separate Al and Fe concentrations 4, 8, and 16 ppm. In the same manner, 1 ml each of 40, 80, and 160 ppm of Al and Fe solutions were added to 10-mg aliquots of alginic acid resuspended in 3 ml of ultrapure water, and the total volume was adjusted to 10 ml to produce samples at 4, 8, and 16 ppm of AlFe = 1:1 (equal weight amount, molar ratio 2:1). pH in all the prepared solutions was confirmed to be 2.7 ± 0.3. Each solution was incubated at 37°C for 1 h with shaking and filtered through a funnel with a teflon perforated plate with No. 5 C filter paper into a 50-ml volumetric flask under vacuum. In order to completely wash off the residual metals absorbed onto the filter paper, filter papers were rinsed with 5 ml of 0.01 mol/l nitric acid and water, and the total volume of the filtrate was adjusted to 50 ml. The quantity of Al and Fe in the solution was measured by atomic absorption spectrometry. The amount of binding was calculated from the amount of residual Al and Fe. The levels of Al and Fe binding under several conditions were also calculated in the same manner as described above using the 1:1 AlFe sample solution, which was prepared by adding 1 ml of each Al and Fe solution to alginic acid, and the sample solutions, which were prepared in the same manner.

3. Measurement of the levels Al and Fe binding to sodium alginate by equilibrium gel filtration method

   A pre-packed PD-10 column with swollen Sephadex G-25 gel (9.1 ml) was utilized. Aluminium oxalate and ferrous oxalate were dissolved in 0.2 mol/l acetic acid/sodium acetate buffer solution, pH 3.5, and the concentrations of Al and Fe were adjusted to 2.5 ppm (Solution A). The column was equilibrated with Solution A. Sodium alginate (20 mg) was dissolved in 10 ml of ultrapure water and added with 10 ml of A'solution (twice the concentrations of the aluminium oxalate and iron (II) oxalate as in Solution A: each metal, 5 ppm). The solution was incubated in a temperature-controlled bath at 37°C for 1 h with shaking, and then 1 ml was aliquoted to load onto the equilibrated column. The elute was collected in 1-ml
fractions in quartz test tubes and 1 ml of concentrated nitric acid was added for decomposition and desiccation. The pellet was resuspended in 4 ml of 0.3 mol/l nitric acid, and the total volume was adjusted to 50 ml for measurement by atomic absorption spectrometry. The amount of metals binding to sodium alginate was calculated from the peak area of a spectrogram against the levels of Al and Fe in the solution as standard values.

Glass, plastic, and quartz instruments were soaked in 20% nitric acid solution overnight and rinsed with ultrapure water prior to use. Each measurement was repeated more than 6 times to obtain the mean value ± standard deviation (SD). The t-test was performed for significance testing with the level of significance of 5%.

RESULTS AND DISCUSSION

The levels of Al and Fe binding to each SDF fraction at pH 3.5 are shown in Fig. 1. In the experiments in the present study, the pH was set at 3.5 or 2.0. The pH 2.0 was assumed to the environment of stomach. The pH 3.5 was selected to make the metal to dissolve sufficiently. Both Fe and Al ions exist stably in this pH range (Delombe et al. 1963). Binding was high in SDF fractions of okra, wakame, and moroheiya. It is known that highly viscous SDF components such as mucopolysaccharides and alginate are present in high quantities in moroheiya (Otani et al. 1995) and okra (Miyazaki 1990) and in wakame (Yamanaka and Ogawa 1998), respectively. We have confirmed for binding of Al and Fe to sodium alginate in our previous experiment that the more viscous the components are, the higher the binding of metals due to physical factors such as viscosity, in addition to chemical factors (Fukushima et al. 2005). Thus, it was assumed that the physical factors of wakame, okra, and moroheiya enhanced the binding capacity of SDF fractions to those metals. In moroheiya, celery and eryngii SDF fractions, both weight and molar concentration of Fe binding amount were higher trend than that of Al.

The levels of Al and Fe binding to each SDF fraction at pH 2.0 are shown in Fig. 2. The levels of metal binding decreased compared to those at pH 3.5; however, the amount of Fe binding in wakame SDF fractions remained almost the same. In our previous study, it was confirmed that sodium alginate was less susceptible to pH effects in less-viscous components (Fukushima et al. 2005). Further investigation will be performed to examine whether this phenomenon explains the result found for wakame in the present study. Only the amount of Al binding in celery measured here was slightly lower than that measured using the colorimetric method of the previous report (Fukushima et al. 2005). This may be due to the inter-

![Graph](image)

Fig. 1. Competitive binding property of Al and Fe ion with SDF (pH 3.5)
Values with asterisk indicate significant difference (p<0.05) in comparison with value of Al and Fe.

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Fig. 2. Competitive binding property of Al and Fe ion with SDF (pH 2.0)
Values with asterisk indicate significant difference (p<0.05) in comparison with value of Al and Fe.

Fig. 3. Competitive binding property of Al and Fe ion with IDF (pH 3.5)
Values with asterisk indicate significant difference (p<0.05) in comparison with value of Al and Fe.

The levels of Al and Fe competitive binding with each IDF fractions are shown in Fig. 3. The levels of both Al and Fe binding were higher in hijiki, wakame, and aloe. In IDF fractions of celery, the amount of Fe binding was especially higher than that of Al. The
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Fig. 4. Competitive binding property of Al and Fe ion with IDF (pH 2.0)
Values with asterisk indicate significant difference ($p<0.05$) in comparison with value of Al and Fe.

Fig. 5. The levels of Al and Fe binding to alginic acid for the presence of Al or Fe alone, or both Al and Fe at an equal amounts
Values with asterisk indicate significant difference ($p<0.05$) in comparison with value of the alone and 1:1 mixture under equal concentrations.

IDF binding was decreased at pH 2.0, as was binding to SDF (Fig. 4). It was indicated that in coexisting solutions of Al and Fe at both pH 2.0 and 3.5, Fe was more likely to bind to IDF than Al in all fiber sources except hijiki and okra (pH 3.5).

In a previous study (Fukushima et al. 2005), it was confirmed that alginic acid has especially superior binding capacity for Al. Thus, the effect of metal concentration on Al binding capacity of alginic acid in the presence of Al and Fe was investigated. The results are shown in Fig. 5. At constant pH, in the presence of both Al and Fe at concentrations of 4 ppm, the amount of Al binding to alginic acid was almost identical to that for Al alone. For concentration increased to 8 and 16 ppm, levels of both Al and Fe binding were significantly increased in the presence of both Al and Fe than for Al or Fe alone. While the amount of Al binding to alginic acid was significantly decreased at pH 2.0 or lower, the amount of Fe binding to alginic acid did not change as much as for Al.

Next, in order to verify these competitive experiments, metal binding experiments using equilibrium gel filtration were conducted. A column packed with gel matrix equilibrated with buffer solution...
containing an equal amount of aluminium oxalate and iron (II) oxalate was loaded with sodium alginate, and eluted with solution containing equal levels of aluminium oxalate and iron (II) oxalate. An example of the calculation of the amount of metal in the eluant is shown in Fig. 6. The area below the equilibrium region indicates the amount of decrease in metal due to binding to alginate. While the amount of Fe binding was 0.9–1.2 mg (1.6×10⁻¹–2.1×10⁻¹ mol) per 1 g of sodium alginate, the amount of Al binding was approximately 0.2–0.3 mg (7.4×10⁻⁴–1.1×10⁻⁴ mol); therefore, it was confirmed that Fe was also more likely to bind to alginate than Al, and the difference was significantly large.

These experimental results revealed that the amount of Al binding to algicin acid decreased at pH 3.5, or lower than that of Fe. This indicates that Al is more likely than Fe to be released and form an Al cation at the pH found in the stomach.

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Effects of pH, Iron and Metal Concentration on Aluminium Coordination


Alの結合に及ぼす鉄、pH、および金属濃度の影響

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著者らはこれまでAl排泄の可能性についてDF画分との結合力の点から検討してきた。その結果、アロソやオクラ、モロヘイヤ、ワカメなどの粘性のある食品から抽出したDF画分に強い金属結合力があることを認めた。そこで、今回はDFとAl強結合力に及ぼすpHとFeおよび金属濃度の影響について検討した。食品から分画したIDF、SDFでは、ビスケイIDF画分以外の両画分ともにAlよりFeの方が結合力が強かった。特にゲルろ過法においてもAlとFeが共存した場合、Feの方がアルミニウム酸と結合しやすいことが確認された。pHを3.5から2.0に下げるとAlおよびFeとも結合力は減少したが、その差はAlの方が大きかった。共存するAlとFeの濃度を順次増加させても、pH2.7±0.3ではアルミニウム酸へAl結合量はわずかに増加しただけだった。

キーワード：アルミニウム、鉄、pH、食物繊維、競合的結合。