Note

Relationship between Physical Parameters of Various Metal Ions and Binding Affinity for Alginate

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We investigated the relationship between the physical parameters of various metal ions, including toxic metal ions, and the binding affinity of these metal ions for alginate (Alg). The binding constant, K , of Sr^{2+} was the highest among all tested metal ions. The order of K values was: $\text{Sr}^{2+} > \text{Pb}^{2+} > \text{Tb}^{3+} > \text{Dy}^{3+} > \text{Ca}^{2+} > \text{Cd}^{2+} > \text{Mg}^{2+} > \text{Fe}^{2+} > \text{Fe}^{3+} > \text{Co}^{2+} > \text{Al}^{3+} > \text{Ni}^{2+} > \text{Cs}^+ > \text{Cu}^{2+} > \text{Ag}^+ > \text{Li}^+ > \text{K}^+$. The metal ions showing the highest K values had ionic radii within the range of about 90–120 pm. Moreover, the K values of divalent or trivalent metal ions tended to be higher than those of monovalent ions. The number of binding sites per 1 mg of Alg (n) was highest for K^+ , followed by Pb^{2+} and Cs^+ . The order of affinity (calculated as the product of n and K) was $\text{Pb}^{2+} > \text{Dy}^{3+} > \text{Tb}^{3+} > \text{Sr}^{2+} > \text{Ca}^{2+} > \text{Mg}^{2+} > \text{Cd}^{2+} > \text{Fe}^{2+}$, $\text{Fe}^{3+} > \text{Cs}^+ > \text{Al}^{3+} > \text{Co}^{2+} > \text{Ni}^{2+} > \text{Cu}^{2+} > \text{Ag}^+ > \text{K}^+ > \text{Li}^+$. Our results support the idea that Alg would be effective as an excretion accelerator and/or absorption inhibitor for various toxic metal ions.

Key words alginate; toxic metal; binding mechanism; affinity

Metal ions absorbed *via* the intestinal tract, respiratory system or skin can cause serious damage to various internal organs, depending on their type and amount. For example, lead (Pb) causes acute damage to the central nervous system, and chronic damage to the kidney and hematopoietic and nervous systems.^{1,2)} Long-term, low-dose exposure to cadmium (Cd) damages the lung (chronic bronchitis, fibrosis, emphysema), kidney (renal tubular degeneration, interstitial inflammation, fibrosis, diabetes, proteinuria), and bone (fake fracture, osteomalacia).³⁾ Cadmium is well known to induce itai-itai disease (kidney tubular damage and osteomalacia),⁴⁾ and moreover, it is carcinogenic.^{3,5)} In addition, aluminum (Al) causes encephalopathy and osteodystrophy,^{6,7)} and copper (Cu) causes hemolytic anemia and gastroenteritis.⁸⁾ In the case of acute oral exposure, damage can be reduced by immediate treatments such as induction of vomiting and/or the use of a laxative to promote excretion of the metal. In addition, chelating agents can be administered; for example, ethylenediaminetetraacetic acid and dimercaptopropionylsulfonate are used as antidotes for Pb poisoning,^{9–11)} and dimercaprol is used for detoxification of Pb and Cu.^{11,12)} However, in the case of chronic exposure, it is essential to use intrinsically safe absorption inhibitors and/or excretion accelerators that are suitable for long-term administration.

Alginate (Alg), an intercellular polysaccharide found in brown algae, is used as a health food and food additive to reduce cholesterol in the blood and to inhibit weight gain.^{13–15)} It also protects the gastric mucous membrane.¹⁶⁾ Moreover, it was reported that accumulation of strontium (Sr) in the human body decreased when sodium alginate (Na-Alg) was ingested before exposure to Sr.¹⁷⁾ Further, when rats pre-fed with Na-Alg for ten days were administered Sr, the cumulative amount of Sr in the body was markedly reduced, compared with the control group.¹⁸⁾ Consequently, it has been suggested that

daily intake of Na-Alg would offer protection against radiation damage from radioactive fall-out by decreasing the absorption of radioactive Sr. Accordingly, the International Atomic Energy Agency (IAEA) has recommended Na-Alg intake for persons exposed to large amounts of radioactive Sr.¹⁹⁾

We have compared the effects of Na-Alg and Ca-Alg in promoting excretion and decreasing absorption of Sr and cesium (Cs) in rats.²⁰⁾ Both additives increased the excretion of Sr, though Cs concentration was significantly reduced only in the Ca-Alg group. There have been some reports describing the capacity of Alg for metal binding and the order of affinity between Alg and metals.^{21,22)} However, there is little information about the relationship between the physical parameters of various metal ions and the binding affinity of these metal ions for Alg. Therefore we performed range and limitation tests of the adapting metals by evaluating the binding constants and the binding amount with Alg using several metal ions in this paper. Moreover, we investigated the relationship between the charge number and the radius of the metals, and their binding affinity. In addition, terbium (Tb^{3+}) and dysprosium (Dy^{3+}) were added as trivalent metal ions to maintain the variation of physical parameters, especially ionic charges and radius.

MATERIALS AND METHODS

Chemicals Na-Alg (high-molecular-weight, rich in guluronic acid) was supplied by Kimica Corporation (Tokyo, Japan). Chlorides of strontium (Sr^{2+}), lead (Pb^{2+}), Tb^{3+} , Dy^{3+} , calcium (Ca^{2+}), cadmium (Cd^{2+}), magnesium (Mg^{2+}), iron(II) (Fe^{2+}), iron(III) (Fe^{3+}), cobalt (Co^{2+}), aluminium (Al^{3+}), nickel (Ni^{2+}), cesium (Cs^+), copper (Cu^{2+}), silver (Ag^+), lithium (Li^+), potassium (K^+) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals and solvents were analytical-grade commercial products.

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Binding Experiments We performed binding experiments according to the method previously reported.²⁰⁾ Aqueous solutions of a metal salt (0, 0.6, 2, 6 or 20 mM) and an equivalent amount containing Na-Alg (2 mg/mL) were mixed. The initial concentrations of the metal ions were set at 0, 0.3, 1, 3 or 10 mM. The mixed solutions (0.5 mL) were incubated at room temperature for 30 min, and then passed through an Amicon Ultra 3k (Merck Millipore, Darmstadt, Germany) at $15000\times g$ for 20 min. The amount of unbound metal in the filtrate was determined using an atomic absorption photometer (ContrAA® 700, Analytik Jena AG, Jena, Germany). The amount of bound metal ion was calculated from the amount of unbound metal ion remaining. Data are expressed as the mean $\pm$ standard deviation (S.D.). The binding constants (K , mM^{-1}) and the number of binding sites per 1 mg of Alg (n , $\mu\text{mol}/\text{mg}$ Alg) were analyzed using double-reciprocal plots. Finally, the affinity of each metal ion for Alg was calculated by multiplying the n and K values.

RESULTS AND DISCUSSION

The relationship between the initial concentration of metal ion and the amount of bound metal ion was examined. The case of Sr^{2+} is shown in Fig. 1 as a typical example. The amount of bound metal ion initially increased concentration-dependently, and was saturated at higher concentrations. The K and n values for Sr^{2+} , calculated from the double-reciprocal plot, were 2046.1 mM^{-1} and $1.52 \mu\text{mol}/\text{mg}$ Alg, respectively (Fig. 1b). Therefore, the affinity ($n \times K$) between Alg and Sr^{2+} was calculated as $3115 \text{ mL}/\text{mg}$ Alg (Fig. 1a).

The values of K , n and affinity for all tested metal ions are listed in Table 1. Among them, Sr^{2+} showed the highest K value, followed by Pb^{2+} , Tb^{3+} and Dy^{3+} . The order of K values was as follows: $\text{Sr}^{2+} > \text{Pb}^{2+} > \text{Tb}^{3+} > \text{Dy}^{3+} > \text{Ca}^{2+} > \text{Cd}^{2+} > \text{Mg}^{2+} > \text{Fe}^{2+} > \text{Fe}^{3+} > \text{Co}^{2+} > \text{Al}^{3+} > \text{Ni}^{2+} > \text{Cs}^{+} > \text{Cu}^{2+} > \text{Ag}^{+} > \text{Li}^{+} > \text{K}^{+}$.

The relationship among K value, charge number and ionic radius is shown in Fig. 2. The metal ions showing the highest K values had ionic radii within the range of about 90–120 pm.

(a)

(b)

Fig. 1. The Relationship between Initial Concentration of Sr^{2+} and the Amount of Sr^{2+} Bound with Na-Alg

The data are the mean $\pm$ S.D. ($n=6$). (a) Normal plot. (b) Double reciprocal plot. C_f , concentration of unbound metal (mM); γ , concentration of bound metal (μmol metal/mg Na-Alg).

Table 1. Values of K , n and Affinity for All Tested Metal Ions

Metal	Symbol	Charge number (+)	Number of binding sites n ($\mu\text{mol}/\text{mg}$ -Alg)	Binding constant K (mM^{-1})	Affinity nK (mL/mg -Alg)
Strontium	Sr	2	1.52	2046.1	3115
Lead	Pb	2	3.59	1906.5	6849
Terbium	Tb	3	1.8	1769	3184
Dysprosium	Dy	3	1.9	1689.7	3205
Calcium	Ca	2	1.25	779.2	977
Cadmium	Cd	2	1.7	398.4	676
Magnesium	Mg	2	1.86	381.9	710
Iron(II)	Fe	2	1.4	376.9	526
Iron(III)	Fe	3	1.85	284.1	526
Cobalt	Co	2	1.3	257.2	334
Aluminium	Al	3	2.03	182.9	371
Nickel	Ni	2	1.55	150.3	233
Caesium	Cs	1	3.4	131.8	448
Copper	Cu	2	1.87	116	217
Silver	Ag	1	1.34	22	29
Lithium	Li	1	1.01	6.4	6.4
Potassium	K	1	6.52	1.07	6.9

Fig. 2. The Relationships between K Value, Charge Number and Ionic Radius of Metal Ions

Moreover, the K values of divalent or trivalent metal ions tended to be higher than those of monovalent ions. It is well known that Alg forms a cross-linked structure with divalent or trivalent metal ions, resulting in gel formation. It appears that metal ions with an ionic radius of about 90–120 pm form robust and water-insoluble gels, whereas, for example, divalent Mg ion with a smaller ionic radius forms only a loose gel with Alg.

On the other hand, the affinity, which is the product of the binding constant and the number of binding sites, is crucial for practical utilization of Alg to absorb metal ions. The number of binding sites per 1 mg of Alg (n) was highest for K^+ , followed by Pb^{2+} and Cs^+ , as shown in Table 1. The calculated values of affinity (nK ; Table 1) were in the following order: $Pb^{2+} > Dy^{3+} > Tb^{3+} > Sr^{2+} > Ca^{2+} > Mg^{2+} > Cd^{2+} > Fe^{2+}$, $Fe^{3+} > Cs^+ > Al^{3+} > Co^{2+} > Ni^{2+} > Cu^{2+} > Ag^+ > K^+ > Li^+$. It is interesting that the affinity between Alg and Cs was relatively small, even though we previously showed that Ca-Alg was effective for promoting excretion and decreasing absorption of Cs in rats.²⁰⁾ Not only the affinity between Alg and metals, but also other factors, such as the specific combination of Alg salt and metal ion, may influence excretion and/or absorption of individual metal ions in the presence of Alg.

People are exposed to various toxic metals in their diet and the environment on a daily basis. Trace levels of Cd are found in rice, a staple of the Japanese diet.^{23,24)} Also, Pb is a contaminant of various foods and food ingredients, especially vegetables.²⁵⁾ Furthermore, the Fukushima nuclear power plant accident in March 2011 resulted in the release of significant amounts of radioactive materials into the atmosphere. Human contamination from these materials, especially radioactive Sr^{2+} and Cs^+ , which have long half-lives, is considered a critical public health issue in Japan. In order to prevent bioaccumulation of such contaminants, many apparently healthy people take so-called “detox foods” on a daily basis. Our present results provide some support for this as a preventive measure. It should be noted that Alg and its salts are very safe and suitable for long-term continued ingestion; indeed, their Acceptable Daily Intake (ADI) evaluated by JECFA: FAO/WHO Expert Committee on Food Additives is given as “Not specified.”

In conclusion, our results indicate that the affinity of Alg for various metal ions is dependent upon their charge and ionic radius. Our results are consistent with the idea that Alg would be effective as an excretion accelerator and/or absorption inhibitor for various toxic metal ions, especially divalent metals such as Pb and Cd. Further studies are planned along these lines.

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Conflict of Interest Chihiro Miyajima and Fumiyoshi Kasahara are employees of Kimica Corporation. The other authors have no potential conflict of interest.

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