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Pharmacological Effects and Utility as a Food Additive of Calcium Alginate

Fumiyoshi Kasahara, Yoko Idota, Yuuki Fukai, Chihaya Kakinuma and Takuo Oghara

Abstract

Here we review the physiological effects of the calcium salt of alginate (Ca-Alg), focusing on our own work. First, we found that Ca-Alg promotes the excretion and decreases the absorption of various metals, and does so more effectively than sodium alginate (Na-Alg). Ca-Alg also reduced plasma cholesterol (Cho) in rats fed a high-Cho diet for 2 weeks. This was considered to be due to reduced intestinal reabsorption of bile acid, resulting from the binding of Ca-Alg and bile acid; this induces an increase of bile acid synthesis from Cho in the liver, leading to a decrease in Cho in plasma. The increase of blood triglyceride (TG) levels in rats fed a high-fat diet for 5 weeks was significantly suppressed by Ca-Alg, leading to decreased fat accumulation in the liver and whole body. Ca-Alg in food was also effective in suppressing the postprandial increase of blood glucose level in rats and humans. An in vitro study suggested that Ca-Alg inhibits the interaction between α-glucosidase and its substrate maltose. In conclusion, Ca-Alg has a number of beneficial effects as a functional food ingredient, and is expected to be a safe and effective food additive for long-term use.

Keywords: calcium alginate, toxic metal, cholesterol, bile acid, triglyceride, micelle, fat, blood glucose level, particle size, noodles

1. Introduction

Alginic acid (Alg) is a polysaccharide derived from algae. Sodium alginate (Na-Alg) is commonly used in foodstuffs as a thickening agent and stabilizer, and is also used as a health food to suppress weight gain and lower blood cholesterol (Cho) [1–4]. In addition, Na-Alg has a protective action on the gastric mucosa [5, 6]. Moreover, when Na-Alg is ingested prior to exposure to strontium (Sr), Sr accumulation in the human body is decreased [7]. However, the sodium salt of Alg can potentially cause hypertension, a major risk factor for dyslipidemia and arteriosclerosis [8]. Therefore, if the calcium salt (Ca-Alg) is as effective as, or superior to, Na-Alg, it might prove to be of greater benefit.

In this chapter, focusing on our own work on Ca-Alg, we will firstly describe how Alg enhances excretion and reduces absorption of Sr and cesium (Cs) in rats. Secondly, we discuss the relationship between the physical parameters of various metal ions and their binding affinity to Alg. Thirdly, we describe the
Cho-lowering effect of Ca-Alg, as well as the reducing effect of Ca-Alg on blood triglyceride (TG) levels, which leads to reduced accumulation of fat in the liver and whole body in rats. Finally, we describe how Ca-Alg in food (noodles) moderates the postprandial blood glucose level in rats and humans and we discuss the mechanism of this effect.

2. Increased excretion and reduced absorption of strontium and cesium

Although several years have passed since the nuclear power plant accident following a severe earthquake in Japan in March 2011, public unease over the possible presence of radioactive materials in foods remains. As we anticipated that foods or medicines containing Alg would help to reduce potential harmful effects, we investigated the effect of Alg on the absorption and excretion of Sr and Cs, whose radioactive isotopes have long half-lives ($^{90}$Sr 28.8 years, $^{137}$Cs 30.17 years). Specifically, we examined and compared the effects of Na-Alg and Ca-Alg on the absorption and excretion of Sr and Cs in rats, as well as investigating their safety [9].

Initially, we examined the adsorption of Sr and Cs by water-soluble Na-Alg. We found that Sr alone was adsorbed by Na-Alg in a concentration-dependent manner, as was Cs alone. On the other hand, when a mixture of Sr and Cs was used, adsorption of Cs by Na-Alg was lower than in the case of Cs alone, whereas adsorption of Sr by Na-Alg was the same as with Sr alone. Thus, both Sr and Cs were concentration-dependently adsorbed by Na-Alg, but adsorption of Cs by Na-Alg was partly blocked in the presence of Sr.

Next, rats were randomized into control (normal diet), Na-Alg, and Ca-Alg groups, and the changes of native Sr and Cs concentrations in plasma were measured after 2 weeks. In the groups fed Na-Alg and Ca-Alg, the Sr concentrations were significantly decreased to 65 and 63% at 1 week, and 77 and 66% at 2 weeks, respectively, compared with the control group. On the other hand, Cs concentration was significantly reduced (to 60% of the control) only at 2 weeks in the Ca-Alg group. Histopathological observation revealed mineral deposition, due to excessive ingestion of sodium, in the pelvic epithelium of the kidney in the Na-Alg group, and epithelial hyperplasia was observed around the deposits. In contrast, no abnormality at all was detected in the Ca-Alg group. These results indicate that Ca-Alg would be safer than Na-Alg if taken daily for protection against radiation damage.

We also randomized rats into control, Na-Alg, and Ca-Alg groups, and administered SrCl$_2$ or CsCl solution. The maximum plasma concentration ($C_{\text{max}}$), time to $C_{\text{max}}$ ($T_{\text{max}}$), and area under the concentration curve for 3 hours after administration (AUC0–3 hours) were calculated from the observed data by subtracting the values before administration. When SrCl$_2$ solution was orally administered in the Na-Alg and Ca-Alg groups, absorption of Sr was significantly decreased in comparison with the control group. In the Ca-Alg group, $C_{\text{max}}$ and AUC0–3 hours were significantly lower than in the control group after oral administration of CsCl solution. No significant difference of Cs plasma concentration profile between the control and Na-Alg groups was observed. Overall, the data suggest that absorption of both Sr and Cs was reduced in the Ca-Alg group, whereas absorption of Cs was not reduced in the Na-Alg group (Figure 1). It should be noted that in these studies we used 10% Na-Alg or Ca-Alg in the diet, whereas 3–4 g/body/day of Alg is typically ingested by humans as a health food [1, 10]. Clinical studies will be required to identify an appropriate level of Ca-Alg for use as a protective agent.
3. Mechanism of binding of alginate and metals

In the case of acute oral exposure to toxic metals, health 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. 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.

Although the effect of Alg on Sr or Cs absorption and excretion has already been reported, there is little information about its effect on other metals. Therefore, 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 Alg [11]. For this purpose, the binding constants (K, mM$^{-1}$) and the binding amount with Alg were evaluated for Sr, Pb, Tl, Dy, Ca, Cd, Mg, Fe(II), Fe(III), Co, Al, Ni, Cs, Cu, Ag, Li, and K. Aqueous solutions of each metal salt and an equivalent amount of Na-Alg were mixed, and the amount of unbound metal remaining in the filtrate was determined using an atomic absorption photometer. The amount of bound metal was calculated from the amount of remaining unbound metal ion, and K values and the number of binding sites per 1 mg of Alg (n) were analyzed using double-reciprocal plots. The affinity of each metal ion for Alg was calculated by multiplying the n and K values. Moreover, the relationships between charge number and radius of these metals and their binding affinity were examined.

The order of K values was as follows: Sr$^{2+}$ > Pb$^{2+}$ > Tb$^{3+}$ > Dy$^{3+}$ > Ca$^{2+}$ > Cd$^{2+}$ > Mg$^{2+}$ > Fe$^{2+}$ > Fe$^{3+}$ > Co$^{2+}$ > Al$^{3+}$ > Ni$^{2+}$ > Cs$^{+}$ > Cu$^{2+}$ > Ag$^{+}$ > Li$^{+}$ > K$^+$. Moreover, metal ions with high K values tended to have ionic radii within the range of about 90–120 pm. On the other hand, the order of affinity for Alg was 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$^+$. The K value and affinity for Alg both tended to be higher for divalent or trivalent metal ions than for monovalent ions. It is well established that Alg forms a cross-linked structure with divalent or trivalent metal ions, resulting in gel formation. These results might indicate that metal ions with an ionic radius of about 90–120 pm form more robust and water-insoluble gels [Figure 2] [11].

Overall, these results indicate 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.

Figure 1. Plasma concentration of Sr (a) or Cs (b) after oral administration of SrCl$_2$ to rats [9]. The data represent means ± S.D. (n = 5).
It is noteworthy that the affinity between Alg and Cs is relatively small, even though we had previously shown that Ca-Alg is effective in promoting excretion and decreasing absorption of Cs in rats [9]. Therefore, not only the affinity between Alg and metals, but also other factors arising from the specific combination of Alg and metal ion, may influence excretion and/or absorption of individual metal ions in the presence of Alg.

4. Cholesterol-lowering effect

Heart disease and cerebrovascular disease are major causes of death in developed countries, accounting for about a quarter of all deaths in Japan [12]. A major factor in their pathogenesis is believed to be dyslipidemia [13–15], which is predominantly a modern lifestyle-related disease [16, 17]. Therefore, there is considerable interest in food additives or health foods that can decrease Cho absorption or promote Cho excretion.

Since Na-Alg reduces the Cho concentration in blood [9], we focused on the Cho-lowering effect of Ca-Alg in rats fed a high-Cho diet [18]. We first examined absorption of the Cho precursor taurocholate by various types of Alg in vitro, using Na-Alg instead of Ca-Alg, since Na-Alg is water-soluble. We found that high-molecular-weight, guluronic acid-rich (HMW-G) Na-Alg showed the greatest adsorption of taurocholate, and so we selected Ca-Alg HMW-G for the following in vivo study.

Rats were fed a high-Cho diet with or without 0.5–2% Ca-Alg for 2 weeks. After 14 days, the plasma concentration of Cho, the portal plasma concentration of bile acid, and the bile acid content in feces were measured. Moreover, in order to monitor safety, blood samples withdrawn after 14 days were used for the measurement of biochemical parameters. In the groups fed the high-Cho diet containing 2% Ca-Alg diet, the plasma concentration of Cho at 2 weeks was significantly lower than that of the group fed high-Cho diet alone. This result was similar to that in the group fed colestimide-containing diet as a positive control. Bile acid excretion in feces tended to increase depending on the concentration of Ca-Alg in the diet. In the group fed the 2% Ca-Alg diet, the portal plasma concentration of bile acid was significantly decreased, compared to that in the high-Cho diet group. Furthermore, the portal

Figure 2.
Relationships between K value, charge number and ionic radius of metal ions [11].
concentration of bile acid was significantly lowered in the 2% Ca-Alg group. There was no significant difference in weight gain or diet intake among the groups during the 2-week experimental period. Microvesicular steatosis was increased in the high-Cho diet and Ca-Alg groups, but remained within the physiological range. While several changes in biochemical parameters and histopathological findings were observed, all values remained within the physiological range (Figure 3).

Overall, these results indicate that Ca-Alg is effective for reducing plasma Cho. A possible mechanism would be enhanced fecal excretion of bile acid due to reduced intestinal reabsorption, which might subsequently stimulate bile acid synthesis from Cho in the liver, leading to a decrease of Cho in plasma (Figure 4).
5. Reduction of blood triglyceride levels

Excess lipid is stored in the form of TG in subcutaneous and internal organs in the body, and is broken down into fatty acids as required [19]. Dyslipidemia, with increased TG and Cho levels in the blood, leads to atherosclerosis, which in turn can lead to cardiovascular disease and stroke. Accumulation of TG can also result in fatty liver disease, leading to decreased hepatic function, liver cirrhosis and potential morbidity, including myocardial infarction, cerebral infarction and angina pectoris, and eventually cancer [20–23].

Since we previously observed that the TG level in blood was decreased by Ca-Alg in rats [18], we next set out to examine the effect of Ca-Alg on elevated TG levels in the blood, hepatic and total body accumulation of fat, and body weight in rats fed a TG-loaded diet for 5 weeks. We also investigated the mechanism of the TG-reducing effect of Alg in vitro [24].

Rats were randomized into five groups: a high-fat diet group (14% w/w lard, HF); 3 Ca-Alg-containing diet groups (2.5, 5 or 10% w/w Ca-Alg) and a resistant maltodextrin (RMD) diet group as a positive control (with 5% w/w RMD). The 10% Ca-Alg group showed a significant reduction of body weight increase from the 7th day. The increase of TG in blood was also significantly suppressed, and the amount of TG excreted in feces was increased. Increase of body fat mass was in the order HF > RMD > Ca-Alg 2.5% > Ca-Alg 5% > Ca-Alg 10%, while the total weight of the extracted fat tissues was significantly reduced in the RMD, 5 and 10% Ca-Alg groups. Hepatic pathology showed clear circular vacuoles apparently representing TG accumulation in the HF group, while fewer vacuoles were seen in the Ca-Alg groups.

These results suggest that Ca-Alg lowers blood TG through direct suppression of TG absorption, independently of its effect on Cho. As regards the mechanism of Ca-Alg action, hepatic pathology showed that clear circular fatty droplets presumed to represent TG accumulation were present in the HF group, but were reduced in...
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the 10% Ca-Alg group. Interestingly, the concentrations of uric acid, allantoin and BUN in plasma were also decreased in all the Ca-Alg groups, though the mechanism involved is unclear (Figure 5).

We then investigated whether Alg affects lipase activity. Na-Alg was suspended in water, and diluted as required. When this solution was added to an emulsion composed of bile acid and lecithin in the presence of lipase, no decrease of lipase activity was observed, ruling out a direct effect on lipase. On the other hand, when Na-Alg was added to an emulsion composed of TG, bile acid and lecithin, the emulsion was well maintained, and a creaming phenomenon was confirmed after 5 days. When water was added to the emulsion, it disintegrated, precipitating lecithin and releasing TG on the liquid surface. These results suggest that Alg stabilizes bile acid micelles containing TG, possibly by absorbing them and forming large micelles that cannot be absorbed, or that are less vulnerable to lipases [24].

These results suggest that Ca-Alg suppresses absorption of TG, leading to reduced blood TG levels, and decreases hepatic and total body accumulation of TG, in addition to promoting excretion. These findings should help to provide a rational basis for designing future clinical trials (Figure 6).

6. Suppression of postprandial blood glucose level

Diabetics may develop serious complications such as retinopathy, nephropathy and neuropathy, in addition to myocardial infarction, cerebral infarction and so on [27, 30], even though the initial subjective symptoms may be minor. Ca-Alg is known to suppress the postprandial increase of blood glucose, and therefore may be helpful in preventing lifestyle-related diseases such as diabetes. Starch is initially decomposed to maltose in the gastrointestinal tract, mainly by α-amylase, before decomposition by α-glucosidase (maltase) to glucose. Transporters located on the cell membrane surface absorb glucose. Ca-Alg should inhibit at least one of these processes to suppress blood glucose levels since it is not absorbed from the gastrointestinal tract. We therefore chose to investigate which of these processes is inhibited by Ca-Alg, and the optimal amount and particle size of Ca-Alg in the diet required to suppress the postprandial increase of blood glucose in rats [25].

We first examined the effect of Ca-Alg concentration on α-glucosidase activity, and observed no significant change compared to the control. On the other hand, the amount of glucose adsorbed on Ca-Alg increased with increasing initial glucose concentration until it reached saturation. The direct binding affinity of glucose for

Figure 6.
A possible mechanism of TG-lowering effect of Ca-Alg [24].
Ca-Alg was low, and the values of the permeation coefficient of glucose showed no significant change. Moreover, it has been reported that the addition of Alg (polysaccharides) increases the viscosity of starch suspension, and there is a positive correlation between apparent viscosity and the decrease of starch digestion [26]. We speculate that Ca-Alg interferes physically with contact between α-glucosidase and maltose by increasing the viscosity of the intestinal contents. It was our assumption that blood glucose suppression by Ca-Alg is the result of decreased efficiency in starch digestion due to the inhibition of α-glucosidase. This may be as a result of increased viscosity of the gastrointestinal contents. We next aimed to define the optimum amount and particle size of Ca-Alg in the diet for the suppression of postprandial blood glucose levels in rats [25]. A diet containing starch together with varying amounts and particle sizes of Ca-Alg was orally administered to rats randomized into five groups: starch with no Ca-Alg (control), or with Ca-Alg (3%; 270 mesh pass, 5%; 270 mesh pass, 5%; 150 mesh pass, or 5%; 80 mesh pass) (n = 3–4 each). Blood was sampled and the glucose level was measured before administration (C₀). Water was added to the five types of starch with or without Ca-Alg and the mixtures were orally administered to conscious rats. Blood glucose levels were measured and the change in blood glucose level (ΔCₜₐ₉) was calculated. Starch containing 5% Ca-Alg (particle size; 270 mesh pass) significantly decreased the ΔCₘₐₓ and ΔAUC, compared to starch containing no Ca-Alg. However, 3% 270-mesh-pass Ca-Alg, or 5% 150- or 80-mesh-pass Ca-Alg produced no significant difference in ΔCₘₐₓ or ΔAUC compared with the 0% Ca-Alg diet (Figure 7) [25].

The in vivo study determined 5% of 270-mesh-pass Ca-Alg to be the most efficient combination of amount and particle size in the suppression of postprandial increases in blood glucose. Compared with 0% Ca-Alg, significant decreases were observed in both ΔCₘₐₓ and ΔAUC, confirming a decrease in both postprandial peak glucose level and the full amount of glucose absorbed within 2 hours of ingestion. It seems likely that the magnitude of action would depend on the surface area of Alg.

Our results support the idea that Ca-Alg increases the viscosity of the gastrointestinal contents, depending upon the surface area of the administered gel. The gel is expected to interfere with the interaction between α-glucosidase and maltose, thereby suppressing the production of glucose, and preventing a sharp rise in blood glucose level. Various products have been reported to moderate glucose absorption; for example, indigestible dextrin has been confirmed to suppress the postprandial increase in blood glucose level by inhibiting α-glucosidase activity [28]. It seems

Figure 7.
Effect of starch diets containing Ca-Alg on blood glucose level in rats [25]. Circles: ΔCₘₐₓ, the difference between the maximum blood glucose level Cₘₐₓ and the pre-feeding blood glucose level C₀. Bars: ΔAUC, the difference between the area under the blood glucose level-time curve from 0 to 120 min after ingestion and the baseline value C₀. The data represent means ± S.D., n = 3 or 4. *p < 0.05, compared with control.
reasonable to consider that Ca-Alg works similarly. Moreover, it was found that 5% of 270-mesh-pass Ca-Alg was the most effective combination of amount and particle size to suppress the postprandial increase of blood glucose (Figure 8).

To analyze the effect of Ca-Alg on the postprandial increase of blood glucose, a prospective, randomized, double-blind, 3-group, 3-phase crossover study was undertaken among healthy Japanese adult subjects [29]. Traditional Japanese udon noodles were selected, and blood glucose levels were measured after ingestion of Ca-Alg–free udon, and noodles containing 5 or 8% Ca-Alg. We also examined the effect of Ca-Alg on other chemical parameters in plasma or serum. Healthy male and female volunteers of 20 years of age or older were divided into three groups so that the average BMI values in the groups were similar. Each group ingested one of the three types of noodles containing 0 (control), 5 or 8% Ca-Alg (weight % to flour and modified starch). Blood was collected by fingertip puncture for blood glucose measurement prior to feeding and after ingestion. The blood glucose level was measured twice at each point using a simple blood glucose meter, and the average value was calculated. After eating the noodles, subjects were given a tasting questionnaire to evaluate “chewiness”, “thickness” and “favorability” of the noodles in a 5-point grading system (Figure 9).

Noodles containing 5 or 8% Ca-Alg caused a significant decrease in ΔC_{max} compared to control noodles. Moreover, ΔAUC also showed a significant decrease in both groups. No significant difference in the time of maximum blood glucose level (T_{max}) was observed among the three groups. This is consistent with previous findings [31] and is similar to findings with α-glucosidase inhibitors, [32, 33] except miglitol [34]. These results indicate that Ca-Alg suppresses the postprandial increase in blood glucose and reduces the total absorption amount of glucose, but without delaying the absorption. Thus, our previous finding that 5% Ca-Alg had a blood glucose-suppressing effect in rats [25] was reproduced in humans.

As for blood biochemical parameters, no significant difference in the amount of Ca change at 30 min after noodle feeding (ΔCa30min) was found between the 5 and 8% Ca-Alg groups compared to the control, but ΔCa at 120 min (ΔCa120 min) showed a significant increase in both groups. In addition, ΔT-Cho30 min showed a slight tendency to decrease in both groups, and ΔT-Cho120 min was slightly decreased in the 8% Ca-Alg group. There was no significant change in other blood test values. We found that the blood Ca concentration at 120 min after eating 5 or 8% Ca-Alg-containing noodles remained within the normal range, 8.5–10.4 mg/dL, [35]
but was significantly increased compared with the value in the control noodle group, suggesting that Ca derived from Ca-Alg was absorbed into the body.

The recommended amount of Ca intake in adults to help prevent diseases such as osteoporosis is 600–900 mg/day.[35] Since the amounts of Ca in noodles containing 8% Ca-Alg and 5% Ca-Alg would be 500 and 320 mg, respectively, about half of the recommended daily intake might be provided by these noodles. The upper limit of tolerable daily Ca intake for Japanese adults is 2500 mg, [35] so even if these noodles were eaten three times a day, the upper limit would not be reached. Thus, the likelihood of excessive Ca intake appears to be low.

Many substances are known to suppress glucose absorption; for example, indigestible dextrin has been shown to inhibit α-glucosidase. Our work showed that Ca-Alg also inhibits α-glucosidase activity [25], and its effect on blood glucose level was similar to or more potent than that of indigestible dextrin [36]. On the other hand, α-glucosidase inhibitors have side effects such as abdominal distention and flatulence. Ingestion of noodles containing 8% Ca-Alg was expected to show an α-glucosidase-inhibitory effect equal to about 1/40th that of a single dose of acarbose [21]. Therefore, it is considered that the likelihood of side effects arising from α-glucosidase inhibition due to ingestion of noodles containing Ca-Alg is extremely low.

Our results raise the interesting possibility that the introduction of food ingredients containing Ca-Alg into the regular diet may be helpful in preventing lifestyle-related diseases, particularly diabetes and osteoporosis, without adversely affecting individual eating habits.

7. Conclusion

Alg, especially Ca-Alg, has a number of beneficial physiological effects. For example, we have shown that Ca-Alg increases excretion and reduces the absorption of toxic heavy metals such as Sr. and Cs in rats. Moreover, Ca-Alg decreases the blood Cho and TG levels, as well as reducing plasma levels of uric acid, allantoin and BUN levels in rats. Further, Ca-Alg moderated the postprandial increase of blood glucose level in rats and humans. Ca-Alg has been confirmed as safe for use as a food additive, and is superior to Na-Alg, because there is no risk of hypertension.
due to increased sodium intake. In addition, Ca-Alg may also have a preventive effect on osteoporosis. Ca-Alg is convenient to take, because it is effective in solid form, and it appears to be suitable for long-term use as an additive or functional food (Figure 10).

Lifestyle-related diseases associated with high calorie intake and insufficient exercise have become a significant social problem [37, 38], and may lead to the development of cancer, heart disease and cerebrovascular disease, which are major causes of death [13–17, 23, 39, 40]. It will be interesting to examine further whether Ca-Alg may also offer potential benefits in relation to lifestyle-related diseases [36].

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Conflict of interest

Fumiyoshi Kasahara is an employee of Kimica Corporation. The other authors have no potential conflicts of interest.
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