Properties of Calcium-Induced Gel Beads Prepared with Alginate and Hydrolysates

Yoshifumi Murata,* Daisaku Jinno, Kyouko Kofuji, and Susumu Kawashima

Faculty of Pharmaceutical Science, Hokuriku University; Ho-3, Kanagawa-machi, Kanazawa 920–1181, Japan.

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Calcium-induced alginate gel beads (Alg-Ca) containing alginate hydrolysate, such as the guluronic acid block (GB), was prepared and the drug release profiles were investigated under simulated gastrointestinal conditions. The addition of GB to Alg-Ca altered its rheological properties. A model drug (hydrocortisone) was incorporated at 78% of its theoretical yield within the dried Alg-Ca containing 5% GB and it was gradually released from the beads in JP XIV 1st medium for disintegration test (pH 1.2), while it was rapidly released with disintegration of the gel matrix in JP XIV 2nd medium (pH 6.8). In contrast, for Alg-Ca containing GB and chitosan, disintegration was not observed in these media and the drug release rate was markedly different. These results demonstrate that the release profiles of drugs incorporated into Alg-Ca can be controlled by adding these polysaccharides.

Key words alginate hydrolysate; alginate gel bead; gel strain; drug release profile

Alginic acid (Alg) is an anionic polysaccharide that consists of α-L-guluronic acid (G) and β-D-mannuronic acid (M). The molecular weight of Alg, the ratio of the two uronic acids (M/G ratio), and the sequence referred to as block structure, are dominant factors in the characterization of the polymer.[1] Three blocks, the G-block (GB), the M-block (MB) and the MG-block are present in the Alg molecule, and these are closely associated with the structure of calcium-induced Alg gel (Alg-Ca), which is formed in the presence of Ca^{2+}. GB is particularly liable to form a Ca^{2+} chelate and Alg with a low M/G ratio generally forms the cured gel matrix.[2] Each block can be obtained by hydrolysis with dilute HCl solution followed by separation by pH-control of the aqueous solution containing these blocks.[3]

An abundant natural polysaccharide, Alg has been used as a medicine as well as a food additive, and it is hoped that Alg-Ca can be used as a vehicle for drug delivery.[4,5] Alg-Ca is able to incorporate a drug into the gel matrix and release it gradually in the gastrointestinal tract after oral administration.[6—8] However, arbitrary control of the drug release rate from Alg-Ca may be difficult because the Alg used to prepare the gel matrix does not necessarily possess the optimum molecular weight, M/G ratio or block structure. Therefore, several attempts have been made to control the drug release profile by modifying the structure of the gel matrix in Alg-Ca.[9—11] We previously reported the effects of polysaccharide additives, such as chitin or chitosan (CS) on drug release from Alg-Ca.[12,13] The additives may modify the structure of gel matrix that holds the drug in Alg-Ca. In the present study, we attempted to prepare Alg-Ca containing Alg hydrolysate and investigated the influence to the gel strength. In addition, the drug release profile from the modified Alg-Ca was investigated under simulated gastrointestinal conditions.

Experimental

Chemicals Alg (sodium salt, degree of polymerization; 450) was obtained from Nacalai Tesque (Kyoto), and CS was obtained from Kimitsu Chem. Industries (CS fine powder, degree of deacetylation, 75—85%; Tokyo). Molecular weight of CS was determined by gel permeation chromatography.[14] Hydrocortisone (HC) was purchased from Wako Pure Chemical Ind. (Osaka). All other reagents used were of analytical grade.

Hydrolysis of Alg Alg was partially hydrolyzed (0.2 M HCl, 2 h, 100 °C), and GB and MB were separated using the method of Haug *et al.*[3] The solutions containing GB or MB were then neutralized with NaOH, followed by precipitation with ethanol and centrifugation (3000 rpm, 5 min). The pellet was washed 3 times with ethanol, and dried *in vacuo* over P_2O_5.

Viscosity of Solution The viscosities of the solutions containing Alg and/or Alg hydrolysate were measured 5 times with a viscometer (VM-1G-M, CBC Materials Co., Ltd., Tokyo) at 37 °C.

Preparation of Modified Alg-Ca Alg-Ca modified by alginate hydrolysate and/or CS was prepared as follows: Alginate was dissolved in distilled and demineralized water with agitation and Alg hydrolysate and/or CS were added to the solution. The model drug, HC (50 mg), was then added to 10 g of solution and mixed until homogeneity. Two grams of this solution was then combined with 10 ml of calcium chloride solution and left to stand for 1 h at room temperature, after which spherical hydrogel beads were obtained. The beads were washed twice with 50 ml distilled water and dried at 30 °C for 8 h on a culture dish, and they were further dried under reduced pressure in a desiccator in the presence of P_2O_5.

Rheological Properties of Alg-Ca The strain (mm) of Alg-Ca pressed (load: 10 g) with a flat adapter (diameter: 15 mm) was measured 3 times with a rheometer (RHEO TEX, SUN Scientific Co. Ltd., Tokyo) at room temperature.

Drug Release Test Release of HC incorporated in Alg-Ca was determined in 500 ml of JP XIV 1st medium (pH 1.2) for 2 h and subsequently in 2nd medium (pH 6.8) for 4 h using JP XIV dissolution test apparatus (Toyama Sangyo, Osaka, paddle method, 150 rpm, 37±0.5 °C). The drug release test was also performed in McIlvain buffer (pH 4.0). Release testing was carried out according to previously reported methods.[13] All dissolution tests were performed in triplicate. If necessary, data were compared using Student's two tailed *t*-test and the difference was considered significant when $p<0.01$.

Results and Discussion

Alg has been used as an additive to increase solution viscosity and degradation of Alg by acid hydrolysis is reflected in the fluidity of an aqueous solution. The viscosity of a 0.5% Alg solution is 19±1 cp (means±S.D., 37 °C), increasing to 52±2 cp with 1% Alg, and 172±3 cp with 2% Alg. However, this marked increase in viscosity is not observed when GB or MB is added to the Alg solution. For example, the viscosities of 1% Alg and 5% GB and of 1% Alg and 5% MB were 82±3 cp and 71±1 cp, respectively.

Figure 1 shows the strain of Alg-Ca (hydrogel) made in the way mentioned in the Experimental section, as determined by a rheometer. It is known that alginate quickly forms stiff gel beads when calcium ions are present in the medium, and the strain of Alg-Ca prepared with 1% Alg became steady at 15 min in the 0.1 M $CaCl_2$. The time course of the strain was

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* To whom correspondence should be addressed. e-mail: y-murata@hokuriku-u.ac.jp

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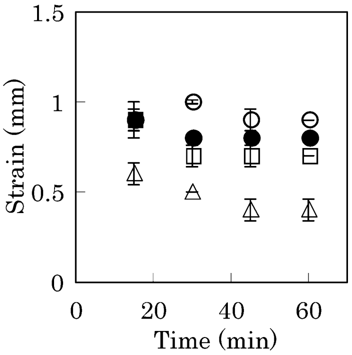

Fig. 1. Changes over Time in the Strain of Alg-Ca (Hydrogel) Prepared with 0.1 M CaCl_2 (Load: 10 g)

○: 1% Alg, ●: 2% Alg, □: 1% Alg+5% MB, △: 1% Alg+5% GB.

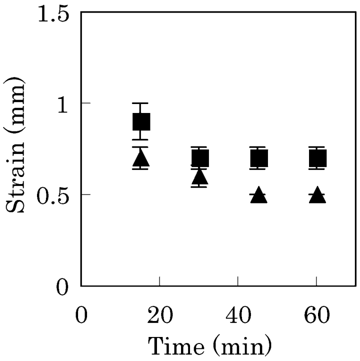

Fig. 2. Effect of Alg Hydrolysate and CS Addition on the Strain of Alg-Ca (1% Alg, Hydrogel) Prepared with 0.1 M CaCl_2 (Load: 10 g)

■: 5% MB+1% CS, ▲: 5% GB+1% CS.

almost the same for Alg-Ca prepared with 2% Alg. On the other hand, the strain of Alg-Ca made from 1% Alg and 5% GB gradually decreased as preparation time increased, and the value was lower than that of 1% or 2% Alg-Ca (hydrolysate free) prepared with the same CaCl_2 concentration. In the case of Alg-Ca prepared with 1% Alg and 5% MB, a pronounced decrease in strain was not observed. This result suggests that GB acts as a component of the Alg gel matrix and reinforces Alg-Ca, although the block was not part of the Alg-molecule. In addition, 5% GB or MB alone did not form cured gel beads and aggregation of the beads was observed. Further addition of 1% CS did not affect the rheological properties of Alg-Ca prepared with a mixture of 1% Alg and 5% hydrolysate (GB or MB), as shown in Fig. 2.

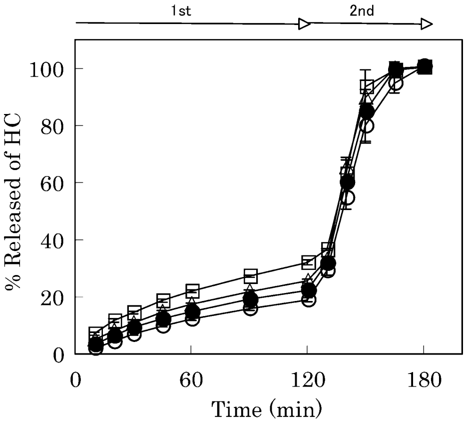

Fig. 3. HC-Release Curves from Alg-Ca* (1% Alg) Containing Alg Hydrolysate Prepared with 0.1 M CaCl_2

* Drug incorporation: 7.7±0.6 mg (n=12). —●—: 5% GB, —○—: 7% GB, —△—: 5% MB, —□—: Alg-Ca (hydrolysate free).

Table 1. Release Parameters of HC in the 1st Medium from Alg-Ca (1% Alg) Containing Alg Hydrolysate Prepared with 0.1 M CaCl_2

Hydrolysate	% Released at 120 min (n=3, mean±S.D.)	$k^{a)}$ (%/min$^{1/2}$) [r^2]
5% GB	22.3±0.9*	2.5 [0.999]
7% GB	19.0±0.7*	2.2 [0.999]
5% MB	25.6±0.7*	2.7 [0.999]
Hydrolysate free	32.1±0.8	3.2 [1.000]

a) Apparent release rate constant. * Significantly different from hydrolysate free at $p<0.01$.

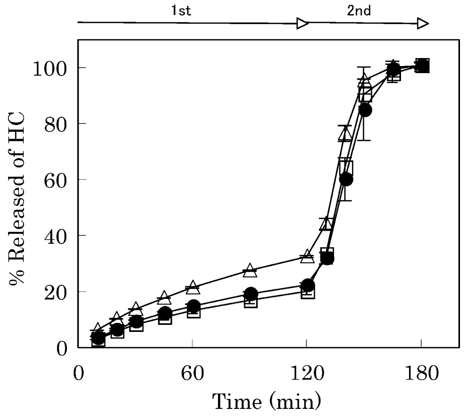

Fig. 4. Effect of CaCl_2 Concentration on HC Release from Alg-Ca* Made from 1% Alg and 5% GB

* Drug incorporation: 7.5±0.3 mg (n=9). —□—: 0.05 M, —●—: 0.1 M, —△—: 0.5 M.

Alg-Ca swelled very slowly and hardly any disintegration was observed in the 1st medium (pH 1.2). Figure 3 shows the HC release profiles of Alg-Ca made from 1% Alg and 5—7% hydrolysate. About twenty percent of HC was released after 120 min in the 1st medium, after which the residual drug was quickly released as the Alg-Ca disintegrated in the 2nd medium (pH 6.8). Table 1 shows the percentage of HC released from the beads and the apparent release rate constant obtained from the linear regression results of fitting the release data (10—120 min) to Higuchi's equation.[15] The addition of Alg hydrolysate suppressed HC-release rate from Alg-Ca, however, the release profile was not affected markedly by the increase in GB concentration. And the release rate constant in the buffer solution (pH 4.0) from Alg-Ca made from 1% Alg and 5% GB was 2.9%/min$^{1/2}$ (r^2=0.953). Figure 4 shows the effect of CaCl_2 concentration on HC release from Alg-Ca. The release rate constants in the 1st medium from the beads prepared with 0.05 M and 0.5 M CaCl_2 were 2.2%/min$^{1/2}$ (r^2=1.000) and 3.4%/min$^{1/2}$ (r^2=1.000), respectively.

It is known that complex formation occurs in Alg solution in the presence of the cationic polymer CS.[16—18] If this phenomenon occurs in Alg-Ca, it may affect the structure of the gel matrix, and, consequently, the drug release profile from Alg-Ca changes. As shown in Fig. 5, Alg-Ca containing CS

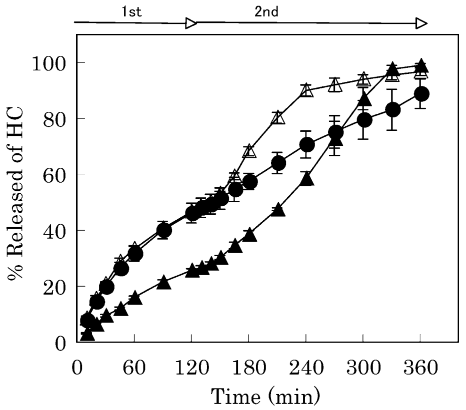

Fig. 5. HC-Release Curves from Alg-Ca (1% Alg) Containing Alg Hydrolysate and CS Prepared with 0.1 M CaCl$_2$

—●—: 1% CS, —▲—: 5% GB+1% CS, —△—: 5% MB+1% CS.

released HC more rapidly than CS-free Alg-Ca in the 1st medium (5.0%/min$^{1/2}$, $r^2=0.998$). However, rapid disintegration of the gel matrix was not observed in the 2nd medium and sustained HC release was observed. And the apparent release rate constant obtained from the HC release data (130—240 min) was 5.6%/min$^{1/2}$ ($r^2=0.993$). In the case of Alg-Ca made from 1% Alg, 5% GB, and 1% CS, about 25% of HC was released after 120 min in the 1st medium, and the release rate was 2.9%/min$^{1/2}$ ($r^2=0.999$). The HC release rates of Alg-Ca made from 1% Alg, 5% MB and 1% CS were 4.3%/min$^{1/2}$ ($r^2=0.999$) in the 1st medium. These results suggest that HC release rate from Alg-Ca in the 1st medium is regulated by Ca^{2+}-Guluronic acid chelate irrespective of whether it arises from Alg or added GB. On the other hand, the rate in the 2nd medium may be affected by complex formation between Alg and CS, which suppresses disintegration of Alg-Ca in the medium.[19,20]

In this study, we prepared Alg-Ca containing Alg hydrolysate, and CS, and investigated the drug release profiles. We observed that addition of GB altered the rheological properties of Alg-Ca, and that the HC release profiles in the gastrointestinal tract may be controlled by modifying the composition of the gel matrix consisting of Ca^{2+}-Guluronic acid chelate or Alg–CS complex. Our results indicate that Alg-Ca shows promise with regard to development of controlled-release drug formulations.

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