Effects of Monovalent Metal Ions and Propranolol on the Calcium Association in Calcium-Induced Alginate Gel Beads

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The effects of monovalent metal ions (K^+ and Na^+) and propranolol ($pK_a = 9.45$, a model cationic drug) on the calcium association in calcium-induced alginate gel beads and on the size changes of the beads were investigated. While calcium ions are an essential component for the gelation of alginate, the associated calcium was discharged by coexisting ionic substances such as the species above without the beads being disintegrated in water and buffers at pHs less than 4.0. The following became evident: (1) the associated calcium was completely discharged at pH 1.0 resulting in contraction of the bead body to about half, (2) at pH 4.0 about 75% of the calcium was lost and the bead volume was greatly reduced as the drug loading increased, (3) the calcium-discharge action was comparable among the above substances, (4) however, the monovalent metal ions brought about expansion of the beads while the drug resulted in contraction of the beads, depending on the bulk concentration of the drug.

Keywords alginate; gel bead; calcium-induced gel; calcium ion; propranolol; monovalent metal ion; calcium dissociation; water migration

Introduction

Alginic acid is a binary heteropolymer consisting of β-D-mannuronic acid(M) and α-L-guluronic acid(G) residues at varying proportions of MM-, GG- and MG-blocks. It has been shown that the GG-blocks are mainly responsible for the selective binding of calcium ions, which contribute to form tight junctions between the GG chains in the gel state.[1,2] Two other blocks, MM- and MG-blocks, take a much lesser part in gelation with calcium ions.[3]

We previously reported the effects of pH and drug concentration on the loading capacity of calcium-induced alginate gel beads for propranolol which was selected as a model cationic drug.[4] The results indicated that the ionization of the carboxy groups in the polymer is significantly involved in the drug loading and in the morphological changes of the gel beads, but little attention was paid to the associated calcium.

The present study is concerned with the effects of the loading of propranolol on the calcium associated with the gelation and the volume of beads in comparison with that of monovalent ions of sodium and potassium.

Experimental

Materials Na-alginate (lot no. AR01, Tokyo Kasei Kogyo, Tokyo) was used after dialysis against distilled water using a Visking cellulose tube (36/32) for 3 d (three water replacements/d) followed by lyophilization. The proportions of GG-, MM- and MG-blocks of the polymer were 24.8%, 37.9 and 37.8, respectively, and the M/G ratio was 1.26.[5] Calcium chloride, dihydrate (special grade, Wako Pure Chem., Osaka) was used. Propranolol–HCl(PRL) (Aldrich Chem. Co., Milwaukee, WI) was used. All other chemicals were of reagent grade.

Preparation of Alginate Gel Beads Alginate gel beads were prepared by dropping the polymer solution (4%(w/w) in distilled water) into a 0.1 M $CaCl_2$ solution, using a peristaltic pump (MP-3, Tokyo Rikakikai, Tokyo) with a polyethylene tubing nozzle (0.50 mm i.d. and 0.80 mm o.d.). The pumping rate was 4 beads/min. The falling height was 3.5 cm. The gel beads were allowed to cure in the $CaCl_2$ solution for more than 3 d and were assumed to be fully cured.[5] Accordingly, the fully-cured gel beads formed contained excess calcium ions which were not associated with the gelation. Fully-cured gel beads were washed once a day for 2 d with freshly distilled water to remove excess calcium ions which are referred to as washed gel beads. The size of the gel beads was determined by measuring their diameter at three different positions and the average value was taken. Bead preparation was conducted at 25 °C throughout.

Procedure Thirty washed gel beads were placed in each of a KCl, NaCl or HCl solution with varying concentrations (0.025—0.5 M) for 2 d at 25 °C. Likewise, washed gel beads of the same number were placed in water and in buffers (10 ml, pH 1.0, KCl–HCl; pH 4.0, acetate) containing propranolol (1—5%(w/w)).

Determination of Calcium Ions in Gel Beads Gel beads of 5—15 were placed in a glass test tube to which 1 ml of nitric acid (61%) was added, and digested on a hot plate at 130 °C for 1 h. After cooling, 1 ml of perchloric acid (70%) was added and the mixture was heated again to 130 °C until the solution became colorless. It is usually accomplished in 1 h. The volume was appropriately adjusted by adding distilled water after cooling. The amount of calcium was then measured by using an atomic absorption analyzer (AA-630-12, Shimadzu, Kyoto) with reference to a calibration curve constructed with known amounts of $CaCl_2$.

Determination of the Amount of Water in Gel Beads Gel beads were dried by heating in an oven at 110 °C for 3 h. The weight difference before and after drying was assumed to be the amount of water in the beads.

Determination of Propranolol Loaded in Gel Beads The drug-loaded gel beads were taken from the drug solution and disintegrated in 5 mM ethylenediaminetetraacetic acid (EDTA)–0.067 M phosphate buffer (pH 7.2). After 24 h standing, the concentration of proplanolol was determined spectrophotometrically at 289 nm.

Results and Discussion

The Amount of Ca^{2+} Associated with the Gelation of Alginate Table I shows the physical dimensions of fully-cured and washed gel beads. The washed beads were slightly swollen compared with the fully-cured beads. There was no difference in the physical appearance between the beads.

Alginate gel beads cured in $CaCl_2$ solution hold a large amount of the bulk medium containing calcium ions which are not associated with the gelation of the polymer. To remove the excess calcium from fully-cured gel beads, the beads were repeatedly washed with distilled water once a day up to 6 d. The spherical integrity of the beads was well kept during washing with a distinct boundary between the surface and the bulk medium.

Table II shows the changes of the amount of calcium in a single bead with repeated washing. The associated amount of the metal ion, M_a, was calculated by

$$M_i = M_a + V_g C_b \tag{1}$$

where M_i was the total amount of calcium. V_g was the

TABLE I. Physical Dimensions of Fully-Cured Gel Beads and Washed Gel Beads

Bead	Radius[a] ($\times 10$ cm)	Volume ($\times 10^3$ cm^3)	Weight[a] (mg/bead)	Water content (%)	Density (g/cm^3)
Fully-cured	1.27	8.49	8.91	92.7	1.05
Washed[b]	1.35	10.2	10.7	94.1	1.05

a) Average value of 15 beads. *b)* Washed gel beads for 2 d by replacing freshly distilled water once a day. The beads were prepared with the initial alginate concentration of 4% (w/w).

TABLE II. Changes of the Amount of Ca^{2+} by Repeated Washing in Fully-Cured Gel Beads

Solution	M_i[a] ($\times 10^7$ mol)	$C_{M,b}$[b] ($\times 10^4$ mol/l)	V_g[c] (μl)	M_a[d] ($\times 10^7$ mol)
0.1 M $CaCl_2$	17.0	842	8.49	9.85
Water[e] day 1	9.58	21.4	8.79	9.39
2	9.26	0.44	9.57	9.26
3	9.08	0.07	9.95	9.08
4	9.00	0.06	9.98	9.00
5	8.98	0.02	10.2	8.98
6	8.95	0.01	10.2	8.95

a) Total amount of Ca^{2+} in a single bead (average value of 15 beads). *b)* Concentration of Ca^{2+} in the bulk. *c)* Volume of water in a single bead calculated from the amount of water (average value of 15 beads). *d)* Amount of Ca^{2+} associated with the gelation in a single bead. *e)* Beads were washed once a day with freshly distilled water.

TABLE III. Effects of K^+, Na^+ and HCl on the Associated Calcium in Washed Gel Beads

Solution	M_i[a] ($\times 10^7$ mol)	$C_{M,b}$[b] ($\times 10^4$ mol/l)	V_g[c] (μl)	M_a[d] ($\times 10^7$ mol)
Water	8.89	0.48	10.1	8.88
0.025 M KCl	6.20	10.2	19.6	6.01
0.05 M KCl	5.23	13.8	20.0	4.95
0.1 M KCl	3.81	16.6	19.8	3.48
0.2 M KCl	2.76	20.2	19.0	2.38
0.5 M KCl	1.73	23.7	17.5	1.31
0.025 M NaCl	6.10	10.4	19.9	5.89
0.05 M NaCl	5.39	13.1	20.5	5.12
0.1 M NaCl	3.79	17.2	20.4	3.44
0.2 M NaCl	2.80	20.5	19.3	2.41
0.5 M NaCl	1.83	23.4	18.0	1.41
0.025 M KCl	0.24	27.0	4.58	0.12
0.05 M KCl	0.11	29.0	5.05	—[e]
0.1 M KCl	0.06	29.0	5.28	—[e]
0.2 M KCl	0.05	29.3	5.23	—[e]
0.5 M KCl	0.04	28.8	5.58	—[e]

a) Total amount of Ca^{2+} in a single bead (average value of 15 beads). *b)* Concentration of Ca^{2+} in the bulk (10 ml). *c)* Volume of water in a single bead calculated from the amount of water (average value of 15 beads). *d)* Amount of Ca^{2+} associated with the gelation in a single bead. *e)* Practically null.

volume of water contained in the beads. C_b was the concentration of calcium in the bulk, which was assumed to be equivalent to the concentration of the unassociated metal ion in the beads.[4] It is apparent that the calcium amount in the bead decreases to an almost constant level accompanied with a slight increase of the volume of the beads.

The M_i equaled the M_a in 2 d, and a very slight liberation of calcium ions from the beads still continued 6 d later. It continues as long as washing with fresh water is repeated. The dissociation rate of the calcium ions however is extremely slow.

The average weights of one droplet of the initial alginate solution and its dried residue were 12.9 and 0.491 mg, respectively. Because $M_i = M_a$ for the beads washed for 2 d, they were assumed to be the washed gel beads in which no free calcium ions remained. The average dry weight of one washed gel bead was 0.539 mg. From this result the amount of calcium associated with the polymer was given as 97.8 mg (2.44×10^{-3} mol)/g of polymer, assuming that the weight difference of the dry portions is solely due to the associated metal. Alternatively, the amount was calculated to be 1.89×10^{-3} mol/g of polymer from the data of day 2 in Table II. Both values agree well.

Effects of K^+, Na^+ and HCl on the Associated Calcium in Washed Beads Table III shows the effects of monovalent ions of potassium and sodium on the associated calcium in the washed gel beads in addition to the effect of the acid or pH. These monovalent ions discharged a large amount of the associated calcium in a hyperbolic manner with their increasing concentration in which their calcium-discharge action was almost identical. The addition of the monovalent ions also caused the bead volume to double. The expansion of the beads seems to have reached its maximum in much lower concentrations of salts than those of the salts examined here, and the migration of water into the beads did not depend on the decrease of the associated calcium. The calcium dissociation would have produced free anionic carboxy groups which electrostatically repel each other and attract more water, but result in little contribution to the bead expansion. These results could be explained as follows: while swelling of the polymer can be regarded as dissolution, the dissolution of the polymer molecules is reduced by the penetration of salt ions, *i.e.* the salting-out effect. To counterbalance this effect and to maintain the structure of the bead consisting of entangled polymer molecules, the beads absorbed more water in a thermodynamically favorable manner. It is considered that the resistance of the network structure was strong enough to keep from further expansion in the range of salt concentration examined. This may be supported by the fact that the spherical shape of the beads was well kept with a clear boundary between the bead surface and the surrounding bulk phase even under higher salt concentrations and the size of the beads remained almost unchanged where the calcium was liberated to a great extent.

In the HCl where the pH changes apparently from 1.8 to 0.71, the remaining calcium in the beads was practically null, and the great contraction of the beads was observed with a loss in their original transparency.[4] The shift of the carboxy groups to a unionized form which is thermodynamically favorable in the acidic environment and complete liberation of the associated calcium result in loss of the electrostatic repelling force, reduction of the polymer dissolution and massive water drainage.

Effect of Propranolol Loading on the Associated Calcium in Washed Beads Table IV shows the changes of the associated calcium in the beads after soaking in the various PRL ($pK_a = 9.45$) solutions. Related data to calculate the associated calcium are also listed. The amounts of the associated PRL were analogously calculated by using Eq. 1.

TABLE IV. Effects of Propranolol Loading on the Associated Calcium in Fully-Cured and Washed Gel Beads

Solution	PRL (%)	M_i[a] ($\times 10^7$ mol)	D_i[b] ($\times 10^7$ mol)	$C_{M,b}$[c] ($\times 10^4$ mol/l)	$C_{D,b}$[d] ($\times 10^3$ mol/l)	V_g[e] (μl)	M_a[f] ($\times 10^7$ mol)	D_a[g] ($\times 10^7$ mol)
0.1 M $CaCl_2$	0	16.7		842		8.19	9.83	
	1	15.5	2.79	776	34.2	7.90	9.41	0.09
	3	14.6	13.0	728	106	7.48	9.17	5.09
	5	14.2	19.6	728	172	7.33	8.84	6.97
Water	0	8.91		0.07		10.3	8.91	
	1	3.66	15.1	14.5	29.8	2.87	3.62	14.2
	3	1.69	27.0	18.5	97.5	3.14	1.63	23.9
	5	1.34	33.2	21.1	165	3.04	1.28	28.2
pH 1	0	0.03		28.4		5.20	—[h]	
	1	0.03	2.31	28.8	35.0	5.07	—[h]	0.54
	3	0.03	6.32	28.4	109	5.17	—[h]	0.68
	5	0.03	9.85	29.2	176	4.74	—[h]	1.51
pH 4	0	2.72		21.2		19.8	2.30	
	1	2.48	10.4	20.6	34.3	14.9	2.17	5.29
	3	1.09	32.4	24.0	100	1.65	1.05	30.8
	5	1.00	37.0	24.5	172	2.03	0.95	33.5

a) Total amount of Ca^{2+} in a single bead (average value of 15 beads). *b)* Total amount of PRL in a single bead (average value of 5 beads). *c)* Concentration of Ca^{2+} in the bulk (10 ml). *d)* Concentration of PRL in the bulk (10 ml). *e)* Volume of water in a single bead calculated from the amount of water (average value of 10 beads). *f)* Amount of Ca^{2+} associated with the gelation in a single bead. *g)* Amount of PRL associated with the polymer in a single bead. *h)* Practically null.

In the $CaCl_2$ solution where a large amount of unassociated calcium still exists in the beads, drug loading caused a minor discharge of the associated calcium, for example, about 90% of the calcium remains at the drug concentration of 5% (169 mM). The associated drug seems to bind to carboxy groups other than the calcium-associated carboxy group. The washed gel beads present a contrast with fully-cured beads. The asssociated calcium was drastically discharged in a hyperbolic manner with increasing drug concentrations. At the same drug concentration (5%), the remaining calcium was only 14%. The associated calcium–drug exchange was highly promoted by removing excess calcium in the beads. At the same time, the size of the beads became contracted two-thirds in radius. The water drainage was probably caused by an electrical neutralization due to a complex formation, possibly an ion-pair formation, between ionized carboxy groups and the drug. It should be noted that the drug loading resulted in the contraction of the beads while the monovalent metal ions brought about the expansion of the beads, although the calcium discharge action was comparable among these substances.

As the apparent pK_a of uronic acid residues in the beads was estimated to be 2.8,[4] changes of the associated calcium were examined at pHs 1.0 and 4.0. At pH 1.0 the associated calcium was entirely discharged regardless of the bulk concentration of the drug and the drug binding was very diminished. The shift of the carboxy group to a unionized form prevails on the association of calcium. At pH 4.0 where the carboxy group should be predominantly ionized, the associated calcium was already reduced to a great extent compared with that in water even in the case of no drug loading, and the beads were swollen about twice as much. As the drug concentration increased, the beads discharged the the associated calcium, contracting extensively in size. Instead, the drug binding greatly increased.

Calcium ions are an essential component for the gelation of alginate. The associated calcium is however discharged by various coexisting substances. In the pH range where the gel beads can maintain their physical integrity as an initially formed sphere, the following became evident: (1) the pH condition was most influential for the associated calcium, for example, at pH 1.0 no associated calcium remained and at pH 4.0 about 75% of the calcium was discharged and further discharged by the loading of PRL, (2) the calcium-discharge action was comparable among the above coexisting substances, suggesting that the charge valence is predominant rather than the chemical species, (3) however, the metal ions expanded the size of the washed gel beads while the drug induced the contraction of the beads, probably due to the formation of an insoluble complex.

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