Acid-Induced and Calcium-Induced Gelation of Alginic Acid: Bead Formation and pH-Dependent Swelling

Toshihisa YOTSUYANAGI,* Isamu YOSHIOKA, Naoki SEGI and Ken IKEDA

Faculty of Pharmaceutical Sciences, Nagoya City University, Mizuho-ku, Nagoya 467, Japan. Received November 7, 1990

The bead formation of alginic acid in the acidic medium (pH 1.0) was investigated in comparison with that of calcium-induced gel beads. The acid-induced gel beads could be visually observed in a few minutes as the transparent spherical figures were detected with a clear boundary between the gel bead and the bulk aqueous phase. The weight change of acid-induced curing beads was as fast as that of the calcium-induced during the initial several hours. But the acid-induced bead lost 15% of its weight at the full stage of gelation, while the calcium-induced lost 37%. The respective beads were incubated at various pHs of 1.0 to 5.0. In the acidic region up to 2.5, the physical integrity of both beads was well maintained. As the pH was raised, they swelled in a sigmoid manner with an inflexion point at about pH 3.2 for the acid-induced beads and at pH 2.8 for the calcium-induced. However, the acid-induced bead disintegrated at a pH of more than 3.5, while the calcium-induced was kept well. The acid-induced beads would be advantageous for some drugs to be incorporated that calcium ions used for alginate gelation might cause undesirable effects such as insoluble salt formation and inactivation due to metal-drug chelation.

Keywords alginic acid; gel bead; calcium-induced gel; acid-induced gel; swelling; pH-dependent disintegration

Introduction

Alginic acid is a binary heteropolymer consisting of β -D-mannuronic acid (M) and α -L-guluronic acid (G) residues at different proportions of MM-, GG- and MG-blocks.¹⁾ It is well known that the GG blocks have a surpassing affinity to divalent metal ions, being responsible for the gelation.²⁾ The gelation involves the cooperative binding of the metal ions, which is described in terms of an "egg box" junction model.³⁾ Calcium association with alginates in the gel-forming reaction has been extensively studied.^{4–6)}

Alginate gel beads contract to a great extent as the medium pH is lowered because of its decreased solubility due to the increasing fraction of unionized carboxy groups and water discharge.^{6,7)} This leads us to the possibility of forming of gel beads without the aid of calcium ions. The calcium-free gel beads would be advantageous for incorporation in some drugs because calcium ions used for gelation might offer undesirable effects such as salt formation and inactivation caused by metal-drug chelation. It is useful, in this respect, to explore the physico-chemical properties of calcium-free gel beads as a possible drug gel vehicle.

The present study is concerned with the gelation behavior and pH-dependent disintegration of acid-induced gel beads in contrast to that of calcium-induced gel beads.

Experimental

Materials Na-alginate (Lot No. AR01, Tokyo Kasei Kogyo, Tokyo) was used after being dialyzed 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 1.28.⁷⁾ Calcium chloride, dihydrate (special grade, Wako Pure Chem., Osaka) was used. All other chemicals were of reagent grade.

Preparation of Calcium-Induced and Acid-Induced Alginate Gel Beads Calcium-induced beads were prepared by the method previously described.⁶⁾ Briefly, an alginate solution (4%, w/w in distilled water) was allowed to fall in drops into 0.1 M CaCl_2 . One droplet of the alginate solution has an average weight of 12.9 mg ($n=20$) with a water content of 96.4%. The resulting gel beads were left in the salt solution for 3 d to become cured; they were then referred to as fully-cured beads. Since the gel beads formed contained excess calcium ions which were not associated with the gelation, the excess calcium was washed out once a day for 2 d

with freshly distilled water. These beads were referred to as washed gel beads. Acid-induced gel beads were prepared in a KCl-HCl buffer (pH 1.0) in the same manner. The beads were also cured in the buffer for 3 d.

Weight Changes of Curing Alginate Gel Beads Ten curing beads were taken out from the salt solution or from the acidic buffer solution at appropriate time intervals and weighed immediately after the removal of surface moisture on a filter paper. The weight fraction of curing beads was expressed as the ratio between the weight of the curing beads and the 10 original droplets of alginate solution assumed at $t=0$.

Determination of Water Content in Alginate Gel Beads Samples of gel beads were dried by heating in an oven at 110 °C overnight. The weight difference before and after drying was assumed to be attributed to the amount of water in the beads, represented as the volume of water assuming $d=1.0$.

Determination of the Size of Alginate Gel Beads The size of the fully-cured beads was determined by taking a photograph of each bead ($\times 20$, 10 beads) and measuring its diameter at three different positions. The average value was taken. Because of the deformed figures of the bead bodies, the volume of the acid-induced bead was measured as follows: 50 beads were placed in a test tube connected with a graduated pipette (1 ml, a minimal graduation 0.01 ml) which was appropriately filled with a buffer (pH 1.0). The difference of the volume with and without the beads was assumed to be the volume of the beads. The average value of two runs was taken.

Results and Discussion

Figure 1 shows the weight changes of curing gel beads in the acidic buffer (pH 1.0) and in 0.1 M CaCl_2 . When a droplet of the alginate solution contacted the acidic buffer solution, the gelation seemed to occur instantly on the surface of the droplet, as observed in the CaCl_2 solution.⁸⁾ The average diameter of the droplet calculated from its weight (assuming the density of the alginate solution 4%, $w/w=1$) was 2.91 mm. In a few minutes, the gel formation could be visually observed as transparent spherical bodies appeared with a distinct boundary between the gel bead and the bulk phase. The results of weight changes indicate that during the initial several hours the interior water is rapidly squeezed out and the curing gradually reaches its full stage in about 1 d in the acid-induced beads and in 2 d in the calcium-induced beads. Thus, the intrinsic rate of acid-induced gelation is likely to be as fast as that of calcium-induced gelation. However, the apparent curing rate of gel beads should be controlled by the penetration rate of calcium ions or hydrogen ions into the interior of

Fig. 1. Weight Fraction Changes of Curing Alginate Gel Beads at 25 °C

△, KCl-HCl (pH 1.0); ○, 0.1 M CaCl₂. W_0 , weight of 10 droplets of initial alginate solution (● assumes $t=0$); W_t , weight of 10 beads at time t . Initial alginate concentration was 4% (w/w).

TABLE I. Physical Dimensions of Gel Beads Formed in 0.1 M CaCl₂ and in KCl-HCl Buffer (pH 1.0)

Bead ^{a)}	Radius ^{b)} (× 10 cm)	Volume (× 10 ³ cm ³)	Weight ^{b)} (mg/bead)	Water reserve ^{c)} (g/g)	Density (g/cm ³)	$M_t^{b,d)}$ (× 10 ⁷ mol)
Calcium-induced						
Fully-cured	1.20	7.15	7.71	12.6	1.08	16.7
Washed	1.28	8.88	9.41	17.5	1.06	11.0
Acid-induced	1.51 (0.99) ^{e)}	10.9 ^{f)}	11.6	26.8	1.06	—

a) Gel beads were always prepared with 4% w/w alginate solution. b) Average value of 10 beads. c) g of water/g of dried residue. d) M_t is the total amount of calcium in a single bead. e) The thickness of flat-shaped beads. f) See Experimental.

the droplets. Therefore, the curing time would depend on the droplet size of the alginate solution used. It should be noted that calcium-induced beads lose 37% of their weight at the full stage of gelation, while the weight loss of the acid-induced bead is only 15%.

While calcium-induced gelation produced almost complete spheres,^{7,8)} acid-induced gelation produced a disc-like bead with a diameter of 1.51 mm and a thickness of 0.99 mm. This indicates that the calcium-induced gel structure which instantly formed on the immediate surface of the droplets is physically strong enough to maintain the spherical figure of droplets. In the mean time, the acid-induced beads seem to gain only a physically frail support of the immediate surface, so that the droplets are deformed by their dead weight. Spherical gel beads could be formed if the droplet size of the polymer solution were reduced.

Table I shows the physical dimensions of the acid-induced and calcium-induced gel beads. The acid-induced gel beads hold more water than the calcium-induced beads, as typically shown in the volume and water reservation data.

Figure 2 shows the changes of water content, i.e. swelling behavior, of the acid-induced and calcium-induced washed gel beads incubated at various pHs of 1.0 to 5.0 for 3 d. The original water content level of both types of the beads before being incubated are pointed out on the ordinate. In the acidic region up to pH 2.5, the physical integrity of the respective beads was well maintained, regardless of the buffer components. As the medium pH was raised, the beads swelled in a sigmoid manner with an inflexion point at about

Fig. 2. Water Content Changes of Alginate Gel Beads Incubated at Different pHs at 25 °C

Acid-induced beads: ○, acetate-HCl; △, KCl-HCl. Calcium-induced beads (washed): ●, acetate-HCl; ▲, KCl-HCl. The water contents of original beads: a) acid-induced beads; b) calcium-induced (washed) bead. Beads were incubated in buffers for 3 d. Each plot represents an average value of 2–3 runs. Initial alginate concentration was 4% (w/w).

pH 3.2 for the acid-induced bead, assuming that the maximal water content was comparable to that of the calcium-induced bead. However, the acid-induced bead disintegrated at a pH of more than 3.5, while the calcium-induced was maintained up to at least pH 5.0 with an inflexion point at pH 2.8.⁷⁾ The inflexion points observed in both types of the beads, which seem to reflect the pK_a values of the carboxy acid residues in the respective beads, were only slightly different from each other, suggesting that the dissociation behavior of the acidic residues was not largely affected by the calcium association. The fully-cured bead incubated at pH 2.8 suffered from a decrease in the associated calcium to 1.36×10^{-7} mol/bead from 8.93×10^{-7} mol/bead obtained before incubation.⁶⁾ The swelling of the gel body is most probably due to the dissociation of carboxy acid residues, which results in more dissolution, namely more water migration into the body. Electrostatic repulsion among the charged residues in the beads is also a possible reason for the swelling.

As previously reported,⁶⁾ no associated calcium remained in the beads when the calcium-induced beads, whether fully-cured or washed were, incubated at pH 1.0. Such a complete discharge of the metal ion was interpreted in a way that the carboxy groups shifted to an unionized form which was thermodynamically favorable in the acidic environment. It is noted that despite no associated calcium in both the beads at pH 1.0, the water content of acid-induced beads was about twice as high as that of calcium-induced beads. These results could be explained as follows: the acid-induced bead was formed with a rather random gel structure as the result of the conversion of uronic acid residues, regardless of M or G, to an unionized form. In contrast, as the calcium-induced bead showed a massive water discharge on its gelation (about one-third in weight), calcium-induced gelation with GG blocks built a very compact and entangled gel structure, which seems to be too strong to be unraveled for expansion, even if the associated calcium were removed.

The acid-induced gel beads are likely to have a disadvantage regarding acid-susceptible drugs when they are incorporated in the beads. However, the beads can maintain their physical integrity up to about pH 3.5, which could allow for a replacement of the original interior water with a lower pH, with water or a drug solution having a

higher pH at which the drug is stable. A primary advantage of acid-induced gel beads is that they are formed under calcium-free conditions and may be useful in avoiding an undesirable calcium-drug interaction.

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