RELATIONSHIP BETWEEN MANNURONIC TO GULURONIC ACID RATIO OF ALGINATE AND CHARGE DENSITY OF Ca-ALGINATE COMPLEX

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Introduction

Calcium-alginate gel is widely used as an immobilization carrier for entrapment of enzymes or whole cells. An alginate molecule is a block co-polymer of D-mannuronic and L-guluronic acid. The gelling is a result of the formation of egg-box junctions consisting of homopolymeric blocks of L-guluronic acid in parallel being held together by divalent cation sources such as calcium^{1, 11)}. Alginate molecules have a wide variety of compositions, namely content of D-mannuronic or L-guluronic acids, according to their origin^{6, 7)}.

M/G ratio, *i. e.*; the mole fraction of D-mannuronic acid (M) to L-guluronic acid (G), is frequently used to characterize alginate composition. M/G ratio of alginate is usually determined with ¹H and ¹³C NMR spectroscopy^{2, 3)}, circular dichroism⁹⁾ or acid hydrolysis followed by liquid chromatography^{4, 5)}. However, these methods require expensive equipment and complicated procedures.

Calcium ion binds preferentially with L-guluronic acid compared to D-mannuronic acid^{8, 11)}. The charge of L-guluronic acid is neutralized by ionic binding with calcium ion. Therefore, the charged state of a calcium-alginate complex is expected to relate to alginate composition. The objective of the present study is to examine the possibility of estimation of M/G ratio of alginate by measuring the charged state of the Ca-alginate complex. The effect of calcium dose on charge density of the Ca-alginate complex was measured by colloid titration.

1. Experimental

1.1 Alginate

Seven kinds of sodium alginate (purchased from Kibun Food Chemifa Co. Ltd) were used. M/G ratios of alginates were reported by the manufacturer to range from 0.21 to 1.05.

1.2 Procedure

A known amount of 0.1 M CaCl solution was added to a sodium alginate solution (220 mg/l). The mixture

was stirred with a magnetic stirrer for 5 min, resulting in formation of a Ca-alginate complex. The charge density of the Ca-alginate complex was measured by colloid titration¹⁰⁾. An excess of N/200 methylglycol chitosan (MGC) was added to the Ca-alginate complex solution resulting in formation of a MGC-Ca-alginate complex. The excess MGC was titrated with N/400 potassium polyvinyl sulfate using toluidine blue as a indicator.

The pH of the sodium alginate solution was adjusted with 0.1 N HCl or 0.1 N NaOH. All the measurements were conducted at 298 K.

2. Results and Discussion

2.1 Charge density of alginate

Figure 1 shows the effect of pH on charge density *Z* of the alginates. The charge density of the alginates decreased rapidly at pH values below 4.0, and was almost constant at pH values above 9.0. Little effect of M/G ratio on charge density of the alginate molecule was observed. These results are consistent with what is expected from the pKa values of alginate, pKa (guluronic acid) = 3.20, pKa (mannuronic acid) = 3.38⁶⁾.

2.2 Charge density of Ca-alginate complex

All the measurements of charge density of the Ca-alginate complexes were carried out at pH 10 because both mannuronic and guluronic acids were considered to be fully dissociated at this pH value. As an example, **Figure 2** shows the effect of Ca-dose on charge density of the Ca-alginate complex when an alginate having an M/G ratio of 0.85 was used. The charge density of the Ca-alginate complex decreased linearly with increasing Ca-dose up to about 27.5 μ mol. With a further increase in Ca-dose, charge density decreased linearly but its slope was much smaller than that of the former line. The relationship between charge density of the Ca-alginate complex and Ca-dose was represented with two straight lines. *Z_i* denotes the charge density of the Ca-alginate complex at the intersection point of the two lines. Interaction between Ca ions and alginate molecules was found to proceed apparently in two steps;

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Fig. 1 Effects of pH and M/G ratio on charge density of alginate

Fig. 2 Effect of calcium dose on charge density of Ca-alginate complex at pH 10.0

namely, a first step in which charge density decreased rapidly from Z_0 to Z_t and a second step in which charge density decreased slowly. Furthermore, the steeper slope of the first line means a stronger affinity of alginate molecules to bind with Ca ions.

In a Ca-alginate gel, the crosslinks are formed by stacking of a homopolymeric sequence of guluronic acid (GG-block) alginate chains mediated by Ca ions^{1, 11)}. It is reported that Ca ions bind with GG-block alginate molecules in preference to homopolymeric sequences of mannuronic acid (MM-chain)^{8, 11)}. The first and second steps in formation of Ca-alginate complexes are considered to reflect interaction between Ca ions and GG-blocks, and between Ca ions and MM-blocks, respectively. Thus, the effect of Ca-dose on charge density of Ca-alginate complexes is expected to relate with the chemical structure of the alginate molecules.

The relationship between Ca-dose and charge density of the Ca-alginate complex can be characterized using two

Fig. 3 Relationship between M/G ratio and Z_t/Z_0

terms; Z_0 and Z_t . Figure 3 shows the relationship between M/G ratio of the alginate and Z_t/Z_0 . M/G ratio of the alginates could be correlated with Z_t/Z_0 . The curve could be expressed by the following relationship within the range of M/G ratio tested.

$$(M/G) = 2.74 (Z_t/Z_0) - 0.216 \quad (1)$$

The correlation is empirical but it can be used for estimation of the M/G ratio of alginate molecules using colloid titration. The method will be helpful to estimate M/G ratios of alginates easily without using expensive equipment or complicated procedures.

Conclusion

The relationship between Ca-dose and the charge density was represented by two straight lines, suggesting different affinity between Ca ions and alginate molecules. M/G ratios of alginates were correlated empirically with Z_t/Z_0 . The correlation may be useful for easy estimation of M/G ratios of alginate molecules.

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