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Calcium binding and calcium-induced gelation of sodium alginate modified by low molecular-weight polyuronate

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ABSTRACT

The functions of low molecular-weight M_w polyuronate on the calcium binding and calcium-induced gelation of normal sodium alginate (ALG) have been investigated. Mannuronate- and guluronate-rich fractions were prepared from ALG at two different M_w for each. In the mixtures of ALG and each alginate fraction, changes in the relative viscosity of dilute solutions and rheological properties of the gels were examined after calcium addition. In dilute solutions, the mannuronate-rich fractions did not substantially alter the calcium binding behavior of ALG regardless of M_w. On the contrary, the guluronate-rich fractions changed the calcium binding behavior of ALG, and more calcium was required for increase in the relative viscosity relating to the formation of egg-box dimers and subsequent aggregations. These results were more evident when M_w of guluronate-rich fractions was lower. Gel rheology was also different between the mannuronate- and the guluronate-rich fractions. In the gels, both fractions decreased the storage modulus in the linear viscoelastic regime with increased yield strain, but these effects of the guluronate-rich fractions were greater than the mannuronate-rich fractions when compared at equivalent M_w. These functions of the guluronate-rich fractions were quite different from those of low-methoxyl pectin fraction. By using well-characterized polyuronate samples, calcium-induced gelation for the mixture of ALG and each low M_w polyuronate was compared on the molecular level.

1. Introduction

Alginate is a collective term for a family of exopolysaccharides produced mainly from the brown seaweeds such as *Laminaria hyperborea* and *Macrocystis pyrifera*. Alginate can be produced in a variety of counterion forms, but sodium alginate is preferentially used as a gelling agent in the food industry mainly due to its high water-solubility. Alginate is a natural polyelectrolyte and made up of a linear copolymer of (1–4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) units (Draget, Moe, Skjåk-Bræk, & Smidsrød, 2006). These units form M-, G-, and MG-block structures depending on the sequence (Moe, Draget, Skjåk-Bræk, & Smidsrød, 1995). G-blocks influence alginate gelation, and in the presence of some multivalent cations, gelation occurs instantly forming ordered structures by cation-bridged intermolecular associations (Gant, Morris, Rees, Smith, & Thom, 1973; Li, Fang, Vreeker, & Appelqvist, 2007). The ordered structures schematically resemble an egg-box, which is now generally accepted as the gelation model of alginate (Djabourov, Nishinari, & Ross-Murphy, 2013; Morris, Rees, Thom, & Boyd, 1978; Thom, Grant, Morris, & Rees, 1982). Formation of egg-box dimers is greatly affected by G-block length in the alginate molecules (Aarstad, Strand, Klepp-Andersen, & Skjåk-Bræk, 2013). Added cations also have effects with calcium the most common among divalent cations for increasing the gel strength (Mørch, Donati, Strand, & Skjåk-Bræk, 2006) mainly because of structural compatibility with G residues. Compared with

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G-blocks, M- and MG-blocks are less sensitive to cations, and the gel strength is less dependent on cation variations (Mørch et al., 2006).

The effects of the macromolecular characteristics of alginate on calcium binding have been investigated (Draget et al., 2001; Funami et al., 2009; Stokke, Smidsrød, Bruheim, & Skjåk-Bræk, 1991). As a molecular parameter, average molecular weight of alginate and content/sequentiality of G residues have been mainly studied (Draget, Skjåk-Bræk, & Smidsrød, 1994; Kong, Kaigler, Kim, & Mooney, 2004; Sikorski, Mo, Skjåk-Bræk, & Stokke, 2007). Recently, the effects of G-block length on calcium binding of alginate were studied using samples which had been enzymatically converted from M to G in the alginate molecules (Aarstad et al., 2013). Furthermore, multiple steps in binding of calcium to alginate were demonstrated using dilute solutions (Borgogna, Skjåk-Bræk, Paoletti, & Donati, 2013; Fang et al., 2007). Gelation and molecular associations of alginate have been extensively of fundamental and practical interest.

Pectin is another representative polyuronate widely used in the food industry. Calcium binding of pectin has been investigated using samples with enzymatic or chemical treatment for modified degree of esterification and average molecular weight. These characteristics influence pectin gelation, including gel strength and the kinetics of gel formation, and have been studied in relation to affinity and sensitivity to calcium (Hotchkiss et al., 2002; Luzio & Cameron, 2008; Ralet, Dronnet, Buchholt, & Thibault, 2001; Thibault & Rinaudo, 1985).

Here we seek to understand the calcium binding and calcium-induced gelation of sodium alginate in the presence of low molecular-weight alginate fractions. Both the M-rich and the G-rich fractions were prepared from normal sodium alginate after acid hydrolysis followed by fractionation by pH. By controlling the hydrolysis condition, two different average molecular-weights were prepared for each fraction. The macromolecular and physicochemical characteristics of these fractions were identified. In the mixtures of normal sodium alginate and each fraction, changes in the relative viscosity of dilute solutions and rheological properties of the gels were examined after calcium addition. Low molecular-weight low-methoxyl pectin fraction was also prepared by two-step enzyme treatment, and the effects were compared to the G-rich fractions at equivalent molecular weight. The objective of the present study is to clarify the functions of low molecular-weight alginate fractions for calcium binding and calcium-induced gelation of normal sodium alginate on a molecular level compared to those of low molecular-weight low-methoxyl pectin fraction. Use of well characterized samples in terms of macromolecular and physicochemical properties must be an essential approach to obtain clear-cut conclusions. However, in previous studies, macromolecular and physicochemical characteristics of polyuronate samples, including average molecular-weight, polydispersity, composition and sequence of monomers etc, are sometimes insufficient, and thus key factors that govern phenomena may be obscure. This point was clarified in the present study. Usefulness of the alginate fractions was also testified as a functional material for increasing the water holding capacity of the gel system as one of the requirements from the industry.

2. Materials and methods

2.1. Materials

Sodium alginate (ALG) SAN-SUPPORT® P-80 and high-methoxyl pectin (HMP) from citrus SAN-SUPPORT® P-160 were provided by San-Ei Gen F.F.I., Inc. (Osaka, Japan) as commercial products. Reagent grade of glucono-δ-lactone (GDL) and calcium carbonate were purchased from Kishida Chemical (Osaka, Japan) as an acidulant and as a water-insoluble calcium source, respectively. The median particle diameter of calcium carbonate was 18.1 μm in the original form, which was pulverized to approx. 10 μm before use. Pectinase (Pectinex® Yield MASH) was purchased from Novozyme (Bagsværd, Denmark) with one unit defined as a capability to liberate 1.0 μmol galacturonic acid per minute at pH 4.0 at 25 °C. Pectin methyl-esterase (Novo shape® XL) was also purchased from Novozyme (Bagsværd, Denmark) with one unit defined as a capability to liberate 1.0 μmol methanol per minute at pH 7.5 at 30 °C.

2.2. Preparation of low molecular-weight alginate fractions

Twenty grams (on a dry base, hereafter the same unless otherwise specified) of ALG was dispersed in 200 ml of 0.3 M HCl and stirred for 17 h at 25 °C. The media were replaced with 50 ml of 0.3 M HCl and was heated at 95 °C for 5 h for hydrolysis. After centrifugation at 750*g* for 15 min, precipitate obtained was dispersed in 50 ml of distilled de-ionized water, and this was repeated twice. The dispersions were adjusted at pH 3.5 using 0.5 M NaOH and stirred at 25 °C for 17 h to recover the G-rich fractions. After centrifugation at 750*g* for 15 min, precipitate obtained was dispersed in 100 ml of distilled de-ionized water, and pH was adjusted at 7.0 using 4.0 M NaOH for solubilization. The solutions were filtered through GF/A glass filters of 1.6 μm pore size and freeze-dried to obtain a sample identified as LM_w-GUL1. On the other hand, supernatant obtained was adjusted at pH 2.6 using 1.0 M HCl and left standing at 25 °C for at least 1 h to recover the M-rich fractions. After centrifugation at 750*g* for 15 min, precipitate obtained was dispersed in 100 ml of distilled de-ionized water, and pH was adjusted at 7.0 using 4.0 M NaOH for solubilization. The solutions were filtered through the glass filters and freeze-dried to obtain a sample identified as LM_w-MAN1. In the procedure above, different heating condition for hydrolysis; for 1 h (in place of 5 h) and different pH conditions for recovery of the G-rich fractions; 3.8 (in place of 3.5) and the M-rich fractions; 2.4 (in place of 2.6) were used to obtain samples identified as LM_w-GUL2 and LM_w-MAN2, respectively.

2.3. Preparation of low molecular-weight low-methoxyl pectin fraction

Thirty grams of HMP was dispersed in 970 ml of distilled de-ionized water and stirred for 30 min at 25 °C. One ml of the pectinase (200 unit/ml) was added to the dispersion, incubated at 40 °C for 2 h for hydrolysis, and heated at 90 °C for 30 min to stop the enzymatic reaction. One ml of the pectin methyl-esterase (45 unit/ml) was then added to the dispersions, incubated at 40 °C for 15 h for de-esterification, and heated at 90 °C for 30 min to stop the enzymatic reaction. The solutions were filtered through the glass filters and freeze-dried to obtain a sample identified as LM_w-LMP.

2.4. Macromolecular and physicochemical characteristics of ALG and alginate fractions

Macromolecular characteristics of ALG and each alginate fraction, including weight-average molecular weight M_w, number-average molecular weight M_n, radius of gyration R_g, and polydispersity index defined by M_w/M_n, were determined by size-exclusion chromatography coupled with a multiangle laser light scattering photometer (SEC-MALS) in basically the same manner as reported (Funami et al., 2009). The Flory exponent ν was also determined based on the relationship between M_w and R_g: