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Rheological investigation of alginate chain interactions induced by concentrating calcium cations

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ABSTRACT

Chain interactions of sodium alginate during its gelation were investigated by a new gelation method which was based on a Ca^{2+}-concentrating gelling process (CCGP) produced by water evaporation of an alginate solution containing $CaCl_2$. For two commercially available sodium alginate samples (low viscosity (LA) and medium viscosity (MA)) having different molecular weight distributions but the same G/M blocks, the critical Ca^{2+} concentrations for their gelation were found to be 4.6 (for LA) and 4.5 (for MA) μmol/mL after evaporating water from 1% of alginate solutions containing 4 μmol/mL of $CaCl_2$. The CCGP gelation method for alginate under the above conditions were confirmed by rheological measurements and the observed highly ordered and uniform mesh structure of the CCGP-formed alginate gels shown in cryo-SEM images. Combinations of LA and MA at different ratios (0:4, 1:3, 2:2, 3:1, 4:0 on weight basis) were studied using the CCGP gelation method to further the understanding of the alginate chain interactions during gelation. Different LA/MA mixtures exhibited different rheological properties in either non-gelled or gelled systems, indicating that the molecular weight distributions of the sodium alginates influence the alginate chain interactions mediated by Ca^{2+}. Thus, an appropriate combination of LA and MA is required for a strong alginate interchain interaction during CCGP, and alginate products with desirable characteristics can be produced by manipulating the mixing ratios of sodium alginates having different molecular weight distributions even at the same total composition and distribution of G/M blocks.

1. Introduction

Alginates, salts of alginic acid, are natural polysaccharides extracted from brown algae (Phaeophyceae). They consist of linear (1–4) linked blockwise copolymers of β-D-mannuronic acid (M) and α-L-guluronic acid (G) (Fischer & Dorfel, 1955; Haug, 1959; Haug & Smidsrød, 1965; Nelson & Cretcher, 1929). The distribution of the blocks MM, GG, MG and GM in the alginate molecule depends on the origin of the alginate including the algae species, the extracted part in the algae, and the algae harvest season (Jorgensen, Sletmoen, Draget, & Stokke, 2007; Smidsrød & Skjåk-Bræk, 1990). Alginates, especially water-soluble sodium alginates, are widely applied in the food and pharmaceutical industries due to their gel-forming properties in the presence of divalent cations such as Ca^{2+} (Brownlee et al., 2005; Josef, Zilberman, & Bianco-Peled, 2010; Li, Hu, Du, Xiao, & McClements, 2011).

An egg-box model was proposed to describe the Ca^{2+}-induced interchain association of alginate molecules. The zigzag-shaped portion of the chains formed by the G molecular fractions in the $^1C_4$ conformation creates pocket-like cavities which can accommodate Ca^{2+} (Grant, Morris, Rees, Smith, & Thom, 1973). Studies found that the formation of the alginate gel junction zones involves dimerization of the polymer chains through the participation of Ca^{2+} as described in the egg-box model (Morris, Rees, Thom, & Boyd, 1978; Sikorski, Mo, Skjak-Braek, & Stokke, 2007), but few studies have verified the egg-box model through direct structural investigation. It has experimentally demonstrated that both the G content and the length of the G block of the alginate molecule contribute greatly to its gel-forming ability and gel strength (Draget, Skjak-Braek, & Smidsrod, 1997; Liu, Qian, Shu, &

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Tong, 2003), but the importance of the MG blocks in the gel formation was also emphasized in later studies (Donati, Holtan, Mørch, Borgogna, & Dentini, 2005; Morch, Donati, Strand, & Skjak-Braek, 2006). Normally, in the presence of Ca^{2+}, G-rich alginates form strong but brittle gels, and M-rich alginates form weak but elastic gels (Izydorczyk, Cui, & Wang, 2005). However, no direct relationship was found between the amount of bound Ca^{2+} and the elasticity of the gel formed by alginates with different M/G ratios (Funami et al., 2009). Thus, the interaction between alginate chains during gel formation still needs further investigation to facilitate practical applications, and the setting of the gelling method is essential to the elucidation of the gelling mechanism.

Currently, dialysis/diffusion (Bellich, Borgogna, Cok, & Cesaro, 2011; Iijima, Hatakeyama, Nakamura, & Hatakeyama, 2002; Li et al., 2011; Rayment et al., 2009; Tu et al., 2005) and internal setting (Funami et al., 2009; Josef et al., 2010; Liu et al., 2003; Lu, Liu, Tong, & Gao, 2006; Straatmann & Borchard, 2003) are commonly used methods for gelling alginate. However, these gelling processes are not easy to control to produce desirable outcomes in terms of the gel properties. In the dialysis/diffusion method, isolated Ca^{2+} cations are diffused into an alginate solution to form heterogeneous alginate-Ca^{2+} gels often saturated with Ca^{2+} cations. Regarding the internal setting method, inert Ca^{2+} cations such as $CaCO_3$ and Ca-EDTA existing in an alginate solution are converted into active Ca^{2+} by slowly changing the pH using glucono-δ-lactone (GDL) to form a Ca^{2+}-limited and homogeneous alginate gel. The former gelling method needs excessive calcium salts, and the latter method needs other chemicals besides alginate and calcium, which make the systems not appropriate for a deep investigation on the alginate chain interactions mediated by Ca^{2+} during the gelling process. In the current study, a new gelling method based on concentrating Ca^{2+} present in an alginate solution through water evaporation was developed, and the alginate chain interactions during the gelling process were investigated without influence of other materials, which can provide a novel insight on the alginate molecular interactions.

2. Materials and methods

2.1. Materials

Two sodium alginates having low and medium viscosity obtained from brown algae (A2158 and A2033) were purchased from Sigma–Aldrich Co. LLC. (St. Louis, MO, United States). Molecular porous membrane tubing (MWCO: 12-14,000) was purchased from Spectrum Laboratories, Inc. (Rancho Dominguez, CA, United States), and sodium 3-(trimethylsilyl) propionate (CAS 37013-20-0) was obtained from Thermo Fisher Scientific, Inc. (Pittsburgh, PA, United States).

2.2. Structural characterization of sodium alginate

The structures of the low-viscosity sodium alginate (LA) and medium-viscosity sodium alginate (MA) were characterized by determining their compositions and molecular weight distributions using gel permeation chromatography (GPC) and ^1H nuclear magnetic resonance (NMR) analysis procedures which are described below:

2.2.1. GPC analysis

The molecular weight distributions of LA and MA were analyzed using the same high-performance intermediate-pressure size exclusion chromatography system equipped with multi-laser scattering and refractive index detectors (HPSEC-MALLS-RI) as described by Zhang (Zhang, Ao, & Hamaker, 2006). LA and MA were purified by dialysis in deionized water using the molecular porous membrane tubing before testing. Using a Na_2SO_4 solution of 0.1 mol/L as the mobile phase, the dissolved LA and MA samples (3 mg/mL) were filtered through a 5-μm filter, and eluted in a flow rate of 1.3 mL/min. The molecular weights of the samples were calculated using a ASTRA 5.3 software (Wyatt Technology Corp., Santa Barbara, CA, United States) and a dn/dc value of 0.165.

2.2.2. ^1H NMR analysis

The compositions of LA and MA were analyzed according to Grasdalen's method (Grasdalen, Larsen, & Smidsrød, 1979) using a Varian Oxford 300 MHz NMR spectrometer (Varian Inc., Palo Alto, CA). LA and MA were partly degraded in a hydrochloric acid solution of pH = 3.0 at 100 °C for 30 min, dialyzed and freeze dried. The pretreated LA and MA samples were repeatedly dissolved in D_2O followed by freeze dried for three times, and then redissolved in D_2O to make a solution of 14 mg/mL for NMR tests at 85 °C. Sodium 3-(trimethylsilyl) propionate was used as a reference for the samples.

2.3. Rheological investigation on alginate gelation induced by concentrating Ca^{2+}

An AR-G2 rheometer (TA instruments, Delaware, USA) equipped with a Peltier plate and a 40-mm diameter steel plate (990285) was used to carry out the rheological investigation. The flow properties of sodium alginate solutions with/without $CaCl_2$ were determined by steady shear tests using a range of shear rates from10 to 200 s^{-1}, and the gelling process of the sodium alginates induced by concentrating Ca^{2+} during water evaporation was monitored using small strain oscillatory shear tests. All the samples were measured with a gap between plates of 1000 μm.

2.3.1. Steady flow tests

Solutions of LA and MA in a range of 0.2–2% (w/v), and solutions with total concentrations of 1% (w/v) and composed of LA/MA at ratios of 0:4 (L0M4), 1:3 (L1M3), 2:2 (L2M2), 3:1 (L3M1) and 4:0 (L4M0) with/without $CaCl_2$ (4 μmol/mL) were prepared. The flow properties were determined at 25 °C by steady shear tests using shear rates in a range from 10 to 200 s^{-1}; the data were collected in a log mode of 10 points/decade.

2.3.2. Frequency sweep tests

Seven replications of each of L0M4, L1M3, L2M2, L3M1 and L4M0 solutions (1% in w/v, 50 mL/sample) with/without $CaCl_2$ (4 μmol/mL) were prepared and stored in 50-mL centrifuge tubes without covers, and placed vertically in a rack with intermittent shaking at 60 °C. A total of 10 samples (a replication of each different solution) were taken out each time when the sample volume in the tubes decreased for about every 5 mL. The water loss percentages of the taken-out samples were tested by weighing the samples before and after the storage at 60 °C, and their rheological properties were tested at 25 °C using frequency sweep tests with frequencies from 0.1 to 10 Hz in their corresponding linear strain range which was determined using strain sweep tests in a range of 0.1–100% strains at 1 Hz and 25 °C.

2.3.3. Time sweep tests

L0M4, L1M3, L2M2, L3M1 and L4M0 solutions (1%, w/v) with/without $CaCl_2$ (4 μmol/mL) were tested at 100 °C using time sweep tests in their corresponding viscoelastic linear strain range which was determined using strain sweep tests in a range of 0.1–100% strains at 1 Hz and 100 °C. The data were collected at a rate of 3 points/min.