Characterization of Amylose Nanogels and Microgels Containing Ionic Polysaccharides

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Shiho Suzuki,†1,2 Junichiro Nishioka,1 and Shinichi Kitamura1
1 Graduate School of Life and Environmental Sciences, Osaka Prefecture University
(1–1 Gakuen-cho, Naka-ku, Sakai 599–8531, Japan)
2 International Polysaccharide Engineering (IPE) Inc.
(Center for R&D of Bioresources, Osaka Prefecture University, 1–2 Gakuen-cho, Naka-ku, Sakai 599–8570, Japan)

Abstract: We prepared and characterized amylose nanogels containing ionic polysaccharides which we used were 4-O-methyl-D-glucurono-D-xylan (GX), alginate, xanthan, and chitosan. Gelation under a shear force followed by a wet pulverization leads to the formation of hybrid nanogels. The resultant nanogels were characterized by particle size analysis, zeta-potential measurement and atomic force microscopy (AFM). Wet pulverization under a pressure of 200 MPa reduced the particle size of the gels from 20–26 μm to 240–670 nm. Zeta potential measurement showed that the ionic polysaccharides increased surface charges of the amylose gels. AFM observations showed the network consisting of submicron size amylose-polysaccharide nano fibrils. The fibrils containing GX were dispersed uniformly, while those containing only amylose were partly aggregated.

Key words: amylose, nanogels, 4-O-methyl-D-glucurono-D-xylan, wet pulverizing system

INTRODUCTION

Amylose, a component of starch, is an essentially linear (1→4)-α-D-glucan. It has been widely studied because of their interesting properties such as gelation, molecular association (retrogradation) and complex formation with guest molecules. However, the use of natural amylose for basic studies on their solution properties contains problems, because of possible contamination with amylopectin to the natural amylose fraction isolated from starch. Furthermore, natural amylose is usually slightly branched. Compared to the native amylose, synthetic amyloses produced by α-glucan phosphorylase are “pure amyloses” which have no branching point in their structure and narrow distributions of molecular weight. These features indicate that the enzymatically synthesized amylose is a standard material of (1→4)-α-D-glucan, and thus, it is an excellent material for a basic research such as a study of solution physics of amylose.

Furthemore, recent development of genetic engineering techniques has been able to create enzymes suited to a mass production. Ezaki Glico Co., Ltd. (Osaka, Japan) and Sanwa Starch Co., Ltd. (Kashiwara, Japan) succeeded in a large scale production of synthetic amylose from inexpensive sucrose or cellulose as the source of glucose-1-phosphate (G-1-P). They applied a synthesis method reported by Waldmann et al.11 This method was either a combination of sucrose phosphorylase and α-glucan phosphorylase or cellulose phosphorylase and α-glucan phosphorylase.22 This method was overcome such disadvantages as high cost of G-1-P and low yields of high-molecular-weight amylose. Now the synthetic amylose is expected to use as a novel functional material.

The gelling properties of amylose using well characterized sample was important to know the mechanism of gelation and aggregation of starch. Basic studies using the synthetic amyloses reported that the properties of amylose in aqueous solution such as aggregation, gelation and film formation has been found to be strongly dependent on its molecular weight.30(31) The amylose with weight average molecular weights ($M_w$) ranging from 100,000 to 200,000 was likely to form gel.

Polysaccharides colloidal nanoparticles have received considerable attention as functional materials in the area of foods, cosmetics and pharmaceutical. Nano-sized particles could give drastic modifications in their properties, as improved mechanical properties and transparency. Recently high-pressure homogenization was applied to prepare nanocrystals of polysaccharides such as cellulose,73 chitin and starch granules.53 In the food industry, particle size reduction of polysaccharide hydrogels was achieved by mechanical homogenization: fluid gels were manufactured by applying a shear force to cooling solutions of agarose during gelation.60(61) However, most gels prepared by this method is micro size.

The nano-sized particles have large surface area, but lowering the stability of their suspensions. To improve the stability of colloidal dispersions in aqueous system, surface charge on the particles could be controlled in different ways, such as variation of pH, addition of polyelectrolyte or introduction of ionic substitution. Colloids with high ze-
ta-potential (negative or positive) are electrically stabilized while colloids with low zeta-potentials tend to coagulate.

In this study, we focused on basic studies for properties of amylose microgels and nanogels using synthesized amylose. We prepared amylose microgels containing ionic polysaccharides to get stability in aqueous solutions, by applying a shear force to cooling solutions. Furthermore, nanogels were prepared from the gels using a wet pulverizing system. This result will lead to the application use of amylose gel to food and pharmaceutical industries.

**MATERIALS AND METHODS**

**Materials.** Synthetic amylose; ESA200K was provided by Ezaki Glico Co., Ltd., 4-O-methyl-D-glucurono-D-xylan (GX) by International Polysaccharide Engineering (IPE) Inc. (Sakai, Japan), and alginate (Alg) (Mw = 182,000) by Kenko Mayonnaise Co., Ltd. (Kobe, Japan). Xanthan gum (Xan) was purchased from Sumitomo Kagaku Industry Co., Ltd. (Yaizu, Japan). The column outlet was connected to a temperature (Star Burst mini HJP-25001H, Sugino Machine Ltd., Osaka, Japan). The column outlet was connected to a Dawn Heleos-II multiaxis laser light scattering photometer (Wyatt Technology Corporation, Santa Barbara, USA) using a silicon RTESP probe (RTESP MPP-11100-10, Veeco Instruments Inc.) operating in a tapping mode.

**Preparation of hybrid gels.** Amylose (2,375 mg) and ionic polysaccharide (125 mg) (0.05 g ionic polysaccharide/g solute) were added in 50 mL of purified water (5% w/v), and heated at 135 °C for 10 min in a TEM-V100 reactor (Taitsu Techno Corporation, Tokyo, Japan). The solution was homogenized by ULTRA-TURRAX T25 digital homogenizer (IKA Werke GmbH & Co. KG, Staufen, Germany) at 15,000 rpm in a chilled beaker. The homogenized gel was stored at 4 °C over night and re-homogenized at 20,000 rpm to prepare 5% slurry. The slurry was diluted 40-fold with purified water (0.125% slurry) and homogenized by the homogenizer at a speed of 20,000 rpm for 1 min. The slurry was filtered with a glass filter (40 μm pore size) and pulverized passing through a wet pulverizing system (Star Burst mini HJP-25001H, Sugino Machine Limited, Uozu, Japan) one time (1 pass) at a chamber pressure of 50, 100, 150, and 200 MPa.

**Dynamic viscoelasticity.** The physical properties of the microgels were studied using a Physica MCR301 rheometer (Anton Paar GmbH, Graz, Austria) equipped with a parallel plate system (25 mm diameter). An aliquot of the slurry (2 mL) was used for the measurement. The frequency sweep measurements of storage modulus (G') and loss modulus (G'') were made at 25 °C over an frequency range of 0.016–15.9 Hz.

**Particle size analysis.** We measured the particle size distributions of micro- and nanogels using two particle size analyzers; Partica LA-950V2 (Horiba Ltd., Kyoto, Japan) and FPAR-1000 (Otsuka Electronics Co., Ltd., Osaka, Japan).

**Zeta-potential measurements.** Zeta-potential of the gels in aqueous solution (pH = 7) were measured by a zeta-potential analyzer (ZEECOM-ZC-2000B, Microwave Co., Ltd., Funabashi, Japan). The gels were diluted 10-3-10-5 fold with purified water and measured. The zeta-potential was calculated using Smoluchowski equation and was averaged of 50 particles.

**Atomic force microscopy.** The gels slurry (0.125% w/v) was diluted 10-3-fold with water, 5 μL of the aliquot was dropped onto a freshly cleaved mica surface, and dried at room temperature over night. The sample was imaged on an atomic force microscopy (AFM) (Nanoscope IIIa scanning probe microscope, Veeco Instruments Inc., Plainview, USA) using a silicon RTESP probe (RTESP MPP-11100-10, Veeco Instruments Inc.) operating in a tapping mode.

**RESULTS AND DISCUSSION**

**Preparation of amylose microgels.**

First, we prepared 5% slurry of amylose gels, and four kinds of hybrid amylose gels contained GX, Alg, Xan, and WSC, respectively (the hybrid gels were denoted to A-GX, A-Alg, A-Xan, and A-WSC, respectively). Figure 1a shows 5% slurry of A-GX prepared under shear force during cooling. All gels were creamy fluid gels. The particle size measurements showed that the sizes of the gels were 19.9–26.0 μm. Further homogenization slightly decreased the particle size of the gels to 14.9–25.1 μm, but not to submicron size (Table 1).

Dynamic viscoelasticity of microgels were measured (Fig. 2). Both G' and G'' increased with increasing frequency. G' and G'' of amylose gels were decreased by adding all four kinds of polysaccharides.

**Preparation of amylose nanogels.**

The microgels were further homogenized using a wet pulverizing system; a star burst system, under a chamber pressure of 50, 100, 150, and 200 MPa. The particle sizes of the gels were decreased as the pressure increased (Fig. 3). Wet pulverizing treatment could decrease the particle size to submicron, and resultant gels prepared under a pressure of 200 MPa were 240–670 nm in size (Table 1). Gels prepared by homogenizer were turbidity dispersions, but gels prepared by wet pulverizing system increased their
transparency (Fig. 1b).

Zeta potential.
The surface charge on the particle is an important character as it determines many properties of the suspensions. The surface charges of amylose gels and hybrid gels in aqueous solution were measured by zeta-potential analyzer (Table 2). The magnitude of zeta-potential is an indication of stability of the colloidal system by electrostatic or charge repulsion or attraction between particles. In general, a large negative or positive zeta-potential of the particles means good dispersion stability. Both of the absolute zeta-potentials of micro- and nanogels of amyloses were low (−3 to 3 mV). These values mean that the particles are less electrophoresed. Whereas, the particle surfaces of A-GX, A-Alg, and A-Xan were negatively charged (−8 to −40 mV) and gels of A-WSC were positively charged. This result suggested that ionic polysaccharides increased charge on the gel surface. The absolute potentials of A-GX and A-Alg were increased by nanomizing of the gels, while those of A-Xan and A-WSC were decreased. Among all the hybrid nanogels, A-GX showed the highest potential (−28.5 mV).

Atomic force microscopy.
Morphology of the nanogels was analyzed by AFM (Fig. 4). AFM images of all gels show gel network consisting of amylose fibrils. Most fibrils were rod-like shape and their size were 100–900 nm in length and 50–200 nm in width, depending on the polysaccharides included in the gels. The height of amylose fibrils were 10–18 nm (Fig. 5). The sizes of the fibrils were almost in accordance with the particle sizes measured by the particle size measurement (240–670 nm). Thus the nanogels were consisted of such rod-like shape nanofibrils. Furthermore, aggregation was observed in amylose gels without containing ionic polysaccharides. The aggregates could reach several micrometers in size. On the other hand, AFM images of A-GX, A-Alg, and A-Xan gels show that the amylose fibrils were dispersed uniformity. In the image of A-WSC, mica surface was covered with a rough film-like network. This could be caused by high molecular weight of WSC. The height profile of aggregates suggested that aggregation of molecular chains were involved, because the height of double helices would be 0.5 nm according to the calculation by molecular dynamics. From these results, it is indicated that amylose nanogels

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**Table 1.** Particle sizes of the gels prepared by homogenizer and wet pulverizing system at various pressures.

| Pressure (MPa)       | Amylose | A-GX | A-Alg | A-Xan | A-WSC |
|----------------------|---------|------|-------|-------|-------|
| Homogenization       | —       | 14900| 22800 | 16800 | 19100 |
| Homogenization and wet pulverization | 50      | 1240 | 640   | 890   | 890   | 1030  |
|                      | 100     | 800  | 430   | ND    | ND    | 790   |
|                      | 150     | 520  | 270   | ND    | ND    | 730   |
|                      | 200     | 440  | 240   | 590   | 320   | 670   |

ND, Not determined.
Particle size of the gels prepared by a wet pulverizer at various pressures. Amylose (◊), A-GX (■), A-Alg (▲), A-Xan (●), and A-WSC (□).

Fig. 3. Zeta potentials of microgels and nanogels in aqueous solution.

| Zeta potential (mV) | Amylose | A-GX | A-Alg | A-Xan | A-WSC |
|---------------------|---------|------|-------|-------|-------|
| Microgels           | 1.5     | -14.5| -11.4 | -32.9 | 15.3  |
| Nanogels            | -2.7    | -28.5| -15.9 | -8.0  | 3.5   |

pH = 7.

Table 2.

AFM images (5 × 5 μm) of nanogels of amylose (A), A-GX (B), A-Alg (C), A-Xan (D), and A-WSC (E).

Fig. 4.

and amylose-ionic polysaccharide nanogels consisted of rod-like shape nanofibrils with 100–900 nm in length, 50–200 nm in width and 10–18 nm in height. The AFM images showed that the fibrils of A-GX were thin compared to those of amylose gels (Fig. 5), which suggests that GX might prevent both the molecular association of amyloses and the aggregation of the fibrils as illustrated in Fig. 6. Amylose nanofibrils aggregated together because of their low surface charges, whereas A-GX nanofibrils dispersed because of electric repulsion by anionic surface charges by GX molecules.

In this study, a shear to amylose-polysaccharide aqueous solution during gelation led to the formation of microgels, and nanogels were successively prepared by wet pulverizing the microgels. AFM measurement revealed that the gels were network consisting of rod-shaped amylose fibrils. The starch nano-unit chains studied by AFM has been reported. The morphology of amylose nano fibrils observed in this study were different from that of amylose aggregates observed by TEM and electron microscopy.

Zeta potential measurements indicated the presence of
ionic polysaccharide on the surface of amylose fibrils. The ionic polysaccharide could be entangled with amylose molecules during gelation and prevent the molecular association of amylose fibrils and improve the stability of the suspensions. Thus, the ionic polysaccharides are predicted to be a good dispersion aids for amylose nanofibrils and GX is particularly effective in the dispersion. The amylose microgels and nanogels prepared in this study could be used in food and pharmaceutical applications.

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