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Complex formation between amylomaize dextrin and *n*-butanol by phase separation system

Jong-Yea Kim, Seung-Taik Lim*

School of Life Sciences and Biotechnology, Korea University, 5-1, Anam-dong, Sungbuk-ku, Seoul 136-701, Republic of Korea

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ABSTRACT

Crystalline dextrin–butanol particles were prepared through a complex formation using separated phases of an aqueous dextrin solution and *n*-butanol. The dextrin concentration and temperature for the complex formation were important parameters to determine the matrix structure of the complex. When dextrin concentration was relatively high (2 or 3%) and the temperature was low (25 °C), amorphous or B-type crystalline particles were produced. However, at a lower dextrin concentration (1%) and a higher temperature (50 or 70 °C), V_H crystalline particles with *d*-spacings of 1.123, 0.657, and 0.429 nm were produced. Mechanical stirring during the complex formation resulted in smaller size (less than 200 nm) and higher yield (more than 50% based on dextrin) of the complex. The complex particles had rectangular shapes with sizes less than 200 nm, and mainly consisted of long and linear dextrins.

1. Introduction

Amylose is a relatively linear polysaccharide composed of α-(1, 4) linked anhydro-glucopyranosyl units. It can self-assemble or crystallize in aqueous media simply by changing the temperature or amylose concentration. Allomorphs of amylose are affected by the solvents used, chain conformation and concentration of amylose, and storage temperature (Gidley & Bulpin, 1989). Cooling a dilute solution containing amylose and a slow diffusion of acetone vapors to amylose solution resulted in the formation of B or A type crystals, respectively (Putaux et al., 2008). In both crystals, 6-folded left-hand amylose double helices are arranged in parallel, but the arrangement pattern of the helices is different. The double helices in the A allomorph are packed in a monoclinic unit cell containing 4 water molecules (Imberty, Chanzy, Pérez, Buléon, & Tran, 1988), whereas those of the B allomorph are packed in a hexagonal unit cell containing 36 water molecules (Imberty & Perez, 1988).

Amylose also forms single helices by accommodating hydrophobic guest molecules inside the helices, which are laterally stacked and form a crystal, the so-called V-amylose. The crystal is classified into several families depending on morphology and electron diffraction diagrams (Cardoso et al., 2007). Hexagonal crystals of V_H type, which results from crystallizing with either fatty acid or hot ethanol (Brisson, Chanzy, & Winter, 1991; Godet, Tran, Delage, & Buléon, 1993; Welland & Donald, 1991; Whittam et al., 1989), have left-handed single helices consisting of 6 anhydroglucopyranosyl units per turn with a pitch of 0.805 nm (Rappenecker & Zugenmaier, 1981). Rectangular crystals, which have a generic name of $V_{butnaol}$, result from the addition of *n*-butanol (Helbert & Chanzy, 1994). Different types of rectangular crystals, which are called $V_{isopropanol}$, can be prepared by the addition of isopropanol or ketones, as well as a large number of complexing agents (Buléon, Delage, Brisson, & Chanzy, 1990; Nuessli, Putaux, Bail, & Buléon, 2003). Square $V_{glycerol}$ crystals, whose diffraction exhibits a near tetragonal symmetry, are formed from high temperature crystallization of amylose with the addition of glycerol (Hulleman, Helbert, & Chanzy, 1996). Also, $V_{naphthol}$ crystals with a tetragonal diffractogram can be obtained with bulky complex agents such as naphthol (Cardoso et al., 2007). It has been considered that $V_{butnaol}$, $V_{isopropanol}$, $V_{naphthol}$ and $V_{glycerol}$ crystals also consist of left-handed 6-fold amylose helices (Booy, Chanzy, & Sarko, 1979; Buléon et al., 1990; Helbert & Chanzy, 1994; Hulleman et al., 1996; Manley, 1964), whereas $V_{naphthol}$ crystal contains 8-fold helices due to the bulky guest compound (Yamashita & Monobe, 1971).

The central cavity of V-amylose single helices is hydrophobic and can host various inorganic and organic molecules (Tomasik, Schilling, & Derek, 1998b, 1998a), so the hosting ability of V-amylose can be applied as targeted and controlled delivery of various substances. Also, the encapsulation of guest compounds by formation of inclusion complexes leads to stabilization of the guest compounds, due to protection against the influence of oxygen or light (Wulff, Avgenaki, & Guzmann, 2005). Thus, molecular inclusions in V-amylose complexes may be used for encapsulation and controlled delivery of various substances in the pharmacology

* Corresponding author. Tel.: +82 2 3290 3435; fax: +82 2 921 0557.
E-mail addresses: tvand@paran.com (J.-Y. Kim), limst@korea.ac.kr (S.-T. Lim).

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and food industry. In addition, application of nanotechnology to the delivery of various biofunctional substances has attracted much attention due to its great potential in improving the efficiency of the bioactive compounds (Chen, Weiss, & Shahidi, 2006). In delivery systems, a comprehensive understanding of physicochemical properties, such as particle size or morphology of the delivery agent, is imperative. Although the physicochemical properties of V-amylose have already been extensively studied, it is not yet fully understood how the size and morphology of V-amylose crystals are affected by the parameters for complex formation such as temperature, chain structure of amylose, concentration of amylose in solution, and the type of guest molecules. V-amylose crystals are normally formed by complex formation with guest compounds as micrometer-sized particles, and overall morphology of the particles is highly dependent on the guest molecules and production method (Lesmes, Cohen, Shener, & Shimoni, 2009). Only a few studies (Kim, Yoon, & Lim, 2009) have been conducted on the preparation of nano-sized V-amylose particles.

In this study, V-amylose complexes were prepared through the interaction between a dextrin in an aqueous solution and *n*-butanol as separated phases. The particle size, morphology, crystalline characteristics, molecular structure and yield of the complex particles were investigated in various physicochemical conditions for the complex formation.

2. Materials and methods

2.1. Materials

Amylomaize starch (Hylon VII, 70% amylose) was provided from National Starch and Chemical Company (Bridgewater, NJ, USA). Isoamylase (EC 3.2.1.68, activity 1122 units/mg) was purchased from Hayashibara Co., Ltd. (Okayama, Japan).

2.2. Preparation of dextrin

A dextrin was prepared following the method of Kim et al. (2009). The amylomaize starch was hydrolyzed in an acid alcohol solution (HCl and ethyl alcohol) at 20 °C for 72 h. The dextrin was purified using an aqueous DMSO solution (90%) and absolute ethyl alcohol (Klucinec & Thompson, 1998). The number-average degree of polymerization (DP_n) of the dextrin was 311 by calculating the ratio between reducing value and total carbohydrate content (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956; Jane & Robyt, 1984; Somogyi, 1952).

2.3. Preparation of complexes

The dextrin (1.0–3.0%, w/v) was dispersed in distilled water (20 mL) and the suspension was heated at 131 °C for 20 min. The dextrin solution was cooled to 70 °C and an aliquot (5 mL) of *n*-butanol was slowly added to the dextrin solution to form a separated butanol phase from the dextrin solution. The dextrin solution was then stirred gently (0–150 rpm) at 25–70 °C for 6 days. At regular intervals, the dextrin solution was collected by centrifugation (20,000 × *g*, 10 min), and then the precipitates were dried at 121 °C. The weight of the precipitates was measured to calculate the yield of the dextrin complex obtained as precipitates. The supernatant was also collected for further analysis of the chain profile of the dextrin that had not been used for complex formation.

2.4. X-ray diffraction pattern (XRD)

At regular intervals during complex formation, aliquots of the dextrin solution were taken and the complex was collected by centrifugation (20,000 × *g*, 10 min). The precipitated complex was then dried in a speed vacuum dryer (N-biotek, Bucheon, Korea) at 40 °C. The crystalline structure of the dried complex was determined using an X-ray diffractometer (MAC Science Co., Japan) at a target voltage and current of 40 kV and 40 mA, respectively. The scanning range and rate were 3–30° (2θ) and 1.0° min^{-1}, respectively.

2.5. Transmission electron microscopy (TEM)

The dextrin complex (0.3 mg) was suspended in distilled water (1 mL), and a drop of the suspension was deposited on a carbon-coated microscopy grid. It was negatively stained with a drop of 2% (w/v) uranyl acetate, dried at room temperature, and imaged using a transmission electron microscope (Philips Tecnai 12, Eindhoven, Netherlands).

2.6. Particle size distribution

The hydrodynamic particle size of the complex particles was determined using a dynamic light scattering detector (Dynapro Titan, Wyatt Technology, Santa Barbara, CA) using a Dynamics program (Version 6.9.2.9, Wyatt Technology, Santa Barbara, CA). The complex particles collected by centrifuging the dextrin solution were re-dispersed in distilled water and the size distribution was analyzed. The refractive index and the viscosity of water, determined using the calculation software, were 1.333 and 1.00 cP at 20 °C, respectively.

2.7. Molecular size of dextrin chains

The molecular sizes of dextrin in the complex and that remaining in supernatant after centrifugation were measured using a high-pressure size exclusion chromatography (HPSEC) with TSK G5,000 PWXL column (Tosoh Bioscience, Montgomeryville, PA, USA), monitored by a refractive index detector (Shodex RI-71, Tokyo, Japan). The free dextrin (dextrin remaining in supernatant) and the dextrin in the complex (about 2 mg, dry basis) were dissolved in 1 N NaOH solution (1 mL) by vortexing, and the solutions were diluted with 50 mM $NaNO_3$ (3 mL). After neutralizing with 1 N HCl, the solutions were autoclaved (121 °C, 20 min) for dissolution, and then filtered through a membrane filter (5.0 μm; Pall Gelman Sciences, Ann Arbor, MI, USA) prior to the HPSEC analysis. Dextran standards of different molecular weights (2,000,000, 500,000, 70,000, and 10,000) were used to calculate the molecular sizes of the dextrins. The mobile phase was a 50 mM $NaNO_3$ solution containing 0.02% NaN_3 and the flow rate was 0.4 mL/min.

The chain profiles of the amylomaize starch and dextrins were measured after debranching with an isoamylase using a high-pressure size exclusion chromatography (HPSEC) with Superdex 75 10/300 GL (Amersham Pharmacia Biotech, Uppsala, Sweden), monitored by a refractive index detector (Shodex RI-71, Tokyo, Japan). The purified dextrin was dispersed in 50 mL of water and then autoclaved at 121 °C for 15 min for dissolution. To debranch the dextrin, an acetate buffer (0.1 M, pH 3.5, 400 μL) and isoamylase (2 μL, 2,900 unit/mL) were added to the dextrin solution, and the mixture was incubated at 45 °C for 24 h. The enzyme was inactivated by boiling the solution for 15 min. The solution was filtered through a glass filter and then filtered again through a membrane filter (5.0 μm; Pall Gelman Sciences, Ann Arbor, MI, USA) prior to the HPSEC analysis. Pullulan standards (112,000, 47,300, 22,800, 11,800, 5,900, and 667) were used to calculate the chain lengths of the dextrins. The mobile phase was distilled water and the flow rate was 0.4 mL/min.