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Formation of crystalline complexes between amylomaize dextrin and ceramide

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ABSTRACT

Complexes between amylomaize dextrin (average DP 311) and ceramide were prepared by using two different blending systems: an aqueous batch system containing ethanol and a two-phase system of isopropyl ether and water. The organic solvents and complex formation temperature (50–90 °C) were important in determining the level of complex formation and its crystalline structure. Under X-ray diffraction analysis, the solvents as well as ceramide could form complexes with dextrin as weak V_{6I} type crystals. However, the crystallinity of complexes was much higher in the presence of ceramide, which would enhance complex formation by forming ternary co-inclusion complexes of dextrin–solvent–ceramide. Compared to the two-phase system, the batch system yielded much higher crystallinity of complexes. With a minor use of ethanol (0.5 mL) in the batch system, aqueous blending of dextrin and ceramide at 50 °C for 2 days followed by a storage at 25 °C for 1 day produced well-defined V_{6I} crystal particles as precipitates. The isolated particles had rectangular shapes with a size of 1 μm or less, and contained about half of the ceramide initially added. The ceramide–dextrin complex exhibited enhanced water dispersibility, up to 45% based on the ceramide content in complex.

1. Introduction

Various bioactive compounds having health-promoting benefits have been attracting industrial interest as functional ingredients in foods, cosmetics and nutraceuticals. Ceramide is one of the natural bioactive components for its skin barrier function and widely used as an additive in cosmetic and pharmaceutical products (Dickson & Lester, 1999; Raith & Neubert, 2000). Structurally, it is a long-chain amino alcohol, covalently linked via an amide linkage to a fatty acyl chain (Cremesti & Fischl, 2000). Several reports have described the benefits of the oral intake of glucosyl ceramide contained in cereal and legumes such as rice, wheat and soybeans as well as sphingomyelin contained in milk. The major benefits of ceramide include improvement of dry skin and alleviation of atopic dermatitis (Asai & Miyachi, 2007; Tsuji et al., 2006).

However, the industrial utilization of ceramide is limited because it is insoluble in water. The hydrophobicity may lead to a poor absorption in the gastrointestinal tract when ceramide is orally administrated (Senkal et al., 2006). Therefore, new formulation or structural modification with ceramide has been required and widely studied to enhance its water solubility and bioavailability. One of potential approaches to increase its water solubility might be the formation of complex with hydrophilic carriers such as cyclodextrin and starch. Recently, genistein–amylose complex was introduced with improved aqueous solubility and bioavailability of genistein (Cohen, Schwartz, Peri, & Shimoni, 2011).

Amylose, a linear starch molecule, tends to form a single helix by accommodating hydrophobic guest molecules inside the helices, which are laterally stacked and form a crystal, the so-called V-amylose. The V-amylose crystals are classified into several families depending on their morphology and electron diffraction diagram (Cardoso et al., 2007). The best documented V-amylose is the V_6 complex, which contains left-handed helices consisting of six D-glycosyl units per turn with a pitch of 0.805 nm (Rappenecker & Zugenmaier, 1981). The structure of this complex is usually induced by the presence of linear molecules such as linear alcohols and lipids (Whittam et al., 1989). With bulky molecules (e.g., naphthol), amylose could form a larger helices consisting of eight D-glycosyl units per turn (Bail, Rondeau, & Buléon, 2005).

The degree of polymerization (DP) of amylose affects the properties of complexes. If the amylose chains are too long, a conformational disorder may be induced, resulting in an imperfection in the crystal structure (Gelders, Vanderstukken, Goesaert, & Delcour, 2004). In contrast, if the amylose chains are too short, crystallization of the complexes can be disturbed. Kim and Lim

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(2009) used dextrins with different degree of polymerizations (DP) prepared by an acidic hydrolysis of a high amylose maize starch in alcohol for the complex formation with *n*-butanol. They found that the complex formation with butanol required only fragment of starch chains (DP_n 311), and thus starch dextrins could form complexes with butanol more efficiently than did intact starch. In addition to the chain length of amylose, the morphology and hydrophobicity of guest molecules also affect the complex formation characteristics with starch or dextrin.

Complex formation with amylose or starch dextrins for various bioactive components (i.e., bioactive long chain FAs such as conjugated linoleic acid and docosahexadecanoic acid) has been extensively studied (Gelders, Goesaert, & Delcour, 2006; Lalush, Bar, Zakaria, Eichler, & Shimoni, 2005). However, research data on the complex formation with ceramide is scarce. Therefore, in this study, the complex formation between a dextrin of amylomaize starch (70% amylose) and ceramide by using batch and two-phase systems was characterized, and the effects of physicochemical conditions on the crystalline structure of complexes were examined.

2. Materials and methods

2.1. Materials

Amylomaize starch (Hylon VII, 70% amylose) was purchased from the National Starch & Chemical Company (Bridgewater, NJ, USA). Ceramide that had been chemically synthesized (>95% purity) was supplied by Doosan Corporation (Kyunggi-do, Korea).

2.2. Preparation of dextrin

A dextrin was prepared following the method of Kim, Yoon, and Lim (2009). Amylomaize starch was hydrolyzed in an acidic alcohol solution (HCl and ethanol) at 20 °C for 72 h. Hydrolyzed dextrins (1 g, dry basis) were purified by dispersing the dextrin in 1 M NaOH solution (10 mL) by vortexing, and the solutions were diluted with distilled water (40 mL). After neutralizing with 1 M HCl (10 mL), the solutions were autoclaved (121 °C, 20 min) before precipitating with 95% ethanol, washing twice with 95% ethanol and once with acetone, and drying at room temperature overnight. The DP_n value of resulting dextrin, calculated as the ratio between total carbohydrate content and the reducing value (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956; Somogyi, 1952), was 311.

2.3. Preparation of complexes

An aqueous solution containing dextrin (250 mg, dry basis) was prepared by dissolving the dextrin in 1 M NaOH solution (2 mL). Distilled water (21 mL) and 1 M HCl solution (2 mL) were then added for neutralization. The resulting solution was purged with nitrogen gas for 3 min to prevent oxidation and autoclaved (121 °C, 20 min). Ceramide (30 mg, dry basis), the guest compound, was dissolved in ethanol (0.5–2.0 mL) under mild stirring at 50 °C and the solution was slowly added to the dextrin solution. The mixture was then stirred vigorously (550 rpm) at 50 °C for 2 days for complex formation.

To compare the complex forming ability with the above-mentioned batch system, another method referred to as two-phase system was investigated. In this system, ceramide (30 mg, dry basis) was dissolved in isopropyl ether (25 and 35 mL, organic phase) under mild stirring at 50 °C and slowly added to the dextrin solution (25 mL, aqueous phase). The two-phases were mixed vigorously (550 rpm) at 50 °C, and the isopropyl ether continually evaporated from the open system during 2 days. After complex formation, the aqueous solution was cooled down to ambient temperature slowly and stored at 25 °C for 1 day. Complexes obtained as precipitates were recovered by centrifuging (5000 × *g*, 15 min) and freeze-drying the solution overnight.

To obtain an in-depth understanding of complex formation behaviors, the crystalline structures and thermal transitions of complexes formed in the batch system were investigated further using the following complex formation conditions: reaction temperature of 50, 70 or 90 °C for 2 days followed by storage at 25 °C for 1 day or 3 days.

2.4. X-ray diffraction pattern

The crystalline structures of the dextrin–ceramide complexes were determined with an X-ray diffractometer (XPERT MPD, Philips Analytical, Almelo, the Netherlands) at a target voltage and current of 40 kV and 30 mA, respectively. The scanning range and rate were 3–30° (2θ) and 1.0°/min, respectively.

2.5. Thermal analysis

The thermal transition of the dextrin–ceramide complexes was determined by differential scanning calorimeter (DSC 6100, Seiko Instruments, Chiba, Japan). The DSC instrument was calibrated with indium, and an empty pan was used as a reference. Freeze-dried complex and water were weighed into an aluminum pan in a 1:2 ratio. The sealed pan was equilibrated in a cold chamber (4 °C, 2 h) before the analysis. The sample was scanned from 40 to 120 °C at a rate of 5 °C/min. The sample was then quickly cooled and reheated under the same conditions for second scanning.

2.6. Quantification of dextrin and ceramide

The re-dispersion of freeze-dried complex (1 mg/10 mL) was autoclaved (121 °C, 20 min) to disrupt any linkages between ceramide and dextrin. The amount of dextrin was quantified using the phenol-sulfuric acid method (Dubois et al., 1956). For the analysis of ceramide, freeze-dried complex (20 mg) were dissolved in an isopropanol/water mixture (7:3, v/v, 10 mL) under a mild stirring at 50 °C for 1 h. The ceramide extracted from the complexes was quantified using a high-performance liquid chromatography (HPLC; Varian Prostar 210, Varian, Palo Alto, CA, USA) with a refractive index detector (Shodex RI-71, Tokyo, Japan). The HPLC-RI system consisted of a pump (P2000, Spectra System, San Jose, CA), an injector valve with a 0.1 mL loop (Rheodyne 7072, Cotati, CA, USA), and a column (TSKgel silica-60, 4.6 mm × 250 mm, Tosoh Bioscience, Montgomeryville, PA, USA). The eluent was an isopropanol/water mixture (7:3, v/v) that had been filtered through a 0.1 μm pore PTFE filter (Whatman, Kent, UK) and the flow-rate of eluent was 0.4 mL/min. Samples were filtered through a membrane filter (5.0 μm pore size, Advantec Inc., Japan) before HPLC analysis.

2.7. Water dispersibility

Water dispersibility of ceramide in the complexes prepared in batch or two-phase system was determined. The freeze-dried complex samples (dry basis, 80 mg) were dispersed in 40 mL of distilled water and the dispersion was magnetically stirred for 3 h at 25 or 50 °C. The dispersion was stored at 25 °C for 24 h and then centrifuged at 5000 × *g* for 15 min to remove the precipitates. The ceramide dispersed in the supernatant was quantified using HPLC as previously described.

2.8. Transmission electron microscopy (TEM)

The morphology of the dextrin–ceramide complexes was observed using a transmission electron microscopy (FEI Technai