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Effects of amylose chain length and heat treatment on amylose–glycerol monocaprate complex formation

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ABSTRACT

Aqueous mixtures of amylose with different chain lengths (DP 23–849), which had been enzymatically synthesized or isolated from potato and maize starches, and glycerol monocaprate (GMC, 5:1 weight ratio) were analyzed by using a differential scanning calorimeter (DSC). The mixtures were thermally treated (first DSC scan: 20–140 °C, 5 °C/min and prolonged heat treatment: 100 °C for 24 h) and its effect on the amylose–GMC complex formation was analyzed by DSC and X-ray diffractometer. The amylose, especially short ones, readily associated in the dispersion forming the amylose–amylose crystals but the presence of GMC inhibited the crystal formation. The longer amylose had the greater possibility for the complex formation with GMC, and the prolonged heat treatment facilitated the amylose–GMC complex formation. Both type I and type II complexes were formed during quenching after the initial DSC heating. However, only the type II complexes were formed after the prolonged heat treatment with improved crystallinity and thermostability.

1. Introduction

The linear amylose chains form single helical complexes with a variety of polar lipids usually in crystal states. The amylose–lipid complex formation is of technological importance in food systems, as it reduces stickiness of the starch-based foods, improves freeze–thaw stability, and retards retrogradation of starch. In this way, amylose may have a potential as a carrier for controlled release of various hydrophobic bioactive compounds (Kim & Lim, 2009).

The formation of amylose–lipid complexes is strongly influenced by thermal history. Less ordered complexes (type I) are assumed to be formed at or below 60 °C when a rapid nucleation occurs (Biliaderis & Seneviratne, 1990; Karkalas, Ma, Morrison, & Pethrick, 1995). However, lamellar-like complexes (type II) are formed when the nucleation is slow and propagation is sufficient by elevating temperature (>90 °C) (Biliaderis & Seneviratne, 1990; Gelders, Vanderstukken, Goesaert, & Delcour, 2004).

Complex formation and its crystalline properties are also influenced by the size of aliphatic lipid and amylose chains. Numerous studies have been conducted on the influence of lipid structure on the complex formation with amylose, reporting that the dissociation temperature of amylose–lipid complexes increased with the length of lipid chain (Eliasson & Krog, 1985; Godet, Bizot, et al., 1995; Godet, Tran, et al., 1995; Tufvesson, Wahlgren, & Eliasson, 2003a; Tufvesson, Wahlgren, & Eliasson, 2003b). The effect of amylose chain length has also been studied by several researchers (Gelders et al., 2004; Godet, Bizot, et al., 1995a). However, the amyloses studied were usually obtained by hydrolyzing starch or by isolating amylose from native starches. Those amyloses were either too short (degree of polymerization, DP < 80) or too long (DP ≥ 400). Recently, the amylose with different chain lengths could be synthesized from sucrose using an amylosucrase from *Neisseria polysaccharea* (NpAS). Thus comprehensive investigation on the influence of amylose chain length is possible. In the present study, glycerol monocaprate (GMC) was used as a ligand for the complex formation with amylose with varied chain lengths, and the influence of amylose chain length on the complex formation was investigated. Additionally, the effect of an extensive heat treatment (100 °C for 24 h) for the aqueous mixture of amyloses and GMC was examined.

2. Materials and methods

2.1. Materials

Potato amylose, GMC, and sucrose were purchased from Sigma Chemical Company (St. Louis, MO, USA). *N. polysaccharea* (ATCC 43768) was purchased from the American Type Culture Collection

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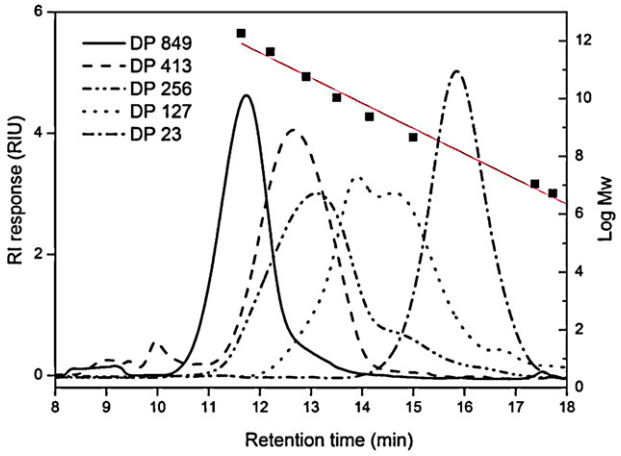

Fig. 1. HPSEC profiles of five amylose with varied chain lengths (DP 23–849) used for complex formation.

(Manassas, VA, USA). Normal maize amylose was isolated from maize starch which was provided by Samyang Genex Company (Seoul, Korea), following the procedure of Jane and Chen (1992). Native normal corn starch was first dispersed in 90% DMSO and precipitate using 95% ethanol. After decanting the supernatant, the precipitate was redissolved in hot water (1.0%, w/v; above 90 °C), and autoclaved at 121 °C for 1 h. The blue screw cap reagent bottle containing the above starch solution was stirred within a boiling water bath for 2 h to completely disperse the starch molecules. n-Butanol (20% by volume) was added, and the solution was stirred at 100 °C for 1 h. The mixture was kept in a Styrofoam box for 24 h at room temperature. The crude amylose–butanol complex was separated by centrifuge (4 °C, 10,000 × *g*, 30 min). Fractionated amylose was further purified by dissolving in DMSO and precipitating using 95% ethanol.

2.2. Amylose synthesis

Recombinant NpAS was cloned and expressed as described previously (Jung et al., 2009). Amylose with varied chain lengths were synthesized by NpAS (400 U/l) from 0.1 M, 0.4 M, and 1.0 M sucrose solutions in 50 mM Tris–HCl buffer solution (pH 7.0) at 35 °C for 18 h (Wang, Kim, Kim, Park, & Yoo, 2011). After the reaction, the mixtures were centrifuged at 5000 × *g* for 10 min, and the precipitate was washed five times with distilled water and then freeze-dried.

The degree of polymerization (DP) of the amylose chains was analyzed by a high-performance size-exclusion chromatography (HPSEC) (Summit HPLC system, Dionex, Sunnyvale, CA, USA) with Shodex OHpak SB-804 and SB-802.5 columns (Showa Denko, Tokyo, Japan) following the procedure of Zhou, Wang, Yoo and Lim (2011). The average DP of each amylose molecule was represented by the apex of the peaks on chromatograms (Fig. 1) which was calculated from the standard curve obtained from glucose, maltotriose, maltopentaose, maltoheptaose and a series of pullulan standards (Showa Denko, Tokyo, Japan) including P5 ($Mw = 5.8 \times 10^3$), P10 ($Mw = 11.8 \times 10^3$), P20 ($Mw = 22.8 \times 10^3$), P50 ($Mw = 47.3 \times 10^3$), P100 ($Mw = 11.2 \times 10^4$) and P200 ($Mw = 21.2 \times 10^4$). AM peak DP was calculated directly from the standard curve. The chain length of amylose could be controlled by using the sucrose solutions of different concentrations: DP 23 from 1.0 M sucrose, DP 127 from 0.4 M sucrose, and DP 256 from 0.1 M sucrose. The maize and potato amyloses used in this study had DP 413, and 849, respectively (Fig. 1).

2.3. DSC sample preparation

GMC (0.4 mg) and amylose (2.0 mg) were dispersed in water (6.0 mg) in an aluminum DSC pan (Seiko Instruments Inc., Chiba, Japan) and hermitically sealed. The control samples were made without GMC or without amylose. These DSC samples were equilibrated at 4 °C for 1 h prior to analysis. To investigate the effect of a prolonged heat treatment, another set of samples were prepared in the same way as mentioned above, and then stored in an oven at 100 °C for 24 h (Tufvesson et al., 2003a). After the prolonged heat treatment, the DSC pans were quench-cooled and then analyzed by DSC.

2.4. DSC analysis

The DSC thermograms were recorded on DSC 6100 (Seiko Instruments Inc., Chiba, Japan). Indium and mercury were used for temperature calibration and an empty pan was used as a reference. All measurements were performed in triplicate. Sample pans were scanned from 20 to 140 °C at a heating rate of 5 °C/min. Samples that were not subjected to a prolonged heat treatment were quench-cooled and immediately rescanned from 20 to 140 °C at a heating rate of 5 °C/min. The melting characteristics of the crystals, including onset (To), peak (Tp), and conclusion (Tc) temperatures, and enthalpy (ΔH) for melting were determined by the EXSTAR6000 Thermal Analysis System (Seiko Instruments Inc., Chiba, Japan).

2.5. X-ray diffraction analysis

GMC (10 mg) and amylose (50 mg) were wetted by 0.5 ml water in an EP tube and stored in an oven at 100 °C for 24 h or heated in an oil bath from 20 °C to 140 °C at a heating rate of 5 °C/min. After the heat treatment, the samples were quench-cooled, dried in a convection oven at 40 °C overnight and ground before analysis. The diffraction patterns were determined by using an X-ray diffractometer (D8 discovery, BRUKER AXS GMBH, Germany), which was operated at 40 mA and 40 kV with the diffraction angles of 3–30° (2θ) and scan speed of 2°/min.

3. Results and discussion

3.1. Thermal transition by initial DSC heating

When an aqueous dispersion of GMC in absence of amylose was heated under a DSC, a sharp GMC melting peak occurred at a temperature around 27 °C with an additional small peak at 92 °C which might be from an impurity (Fig. 2). The control samples containing absolute amylose displayed an endothermic peak for the melting of amylose crystals which was very much different according to the chain length of amylose (Fig. 3A-1). The melting temperature increased but melting enthalpy decreased with the increase in amylose chain length. The smaller amylose chains tended to associate more readily during enzyme synthesis reaction but the resulted crystals melted at lower temperature. However, these synthetic amyloses exhibited typical B-type XRD patterns (indicated by the peak at around 17°) with similar intensities (Fig. 4A). No obvious endothermic peak was observed for the large amylose chains isolated from maize and potato starches (DP 413 and 849). It indicates that the isolation procedure from native starches resulted in the amorphous structure of amylose (Fig. 4A).

Fig. 3A-2 shows the thermograms of the aqueous mixtures of GMC and amyloses (1:5 weight ratio). The large peak around 27.5 °C is responsible for the melting of GMC (Fig. 3A-2). The mixture samples, except that containing potato amylose (DP 849), exhibited thermograms similar to those of pure amylose samples, although the small impurity peak of GMC appeared for all thermograms. The