Characteristics of Amylose Forming Complexes with Glycerol Monooleate and Monostearate

(Received April 12, 2002; Accepted August 19, 2002)

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Abstract: The formation of amylose complexes with glycerol monooleate (MG-C18:1) or glycerol monostearate (MG-C18:0) was conducted in aqueous solution using a commercial amylose with an average degree of polymerization ($\overline{DP}$) of 18. The chain distributions of amylose precipitated by forming a complex in aqueous solution (20°C) were analyzed by high-performance anion exchange chromatography with a pulsed amperometric detector (HPAEC-PAD). The critical $\overline{DP}$ s of amylose forming complexes with MG-C18:1 and MG-C18:0 were $\overline{DP}$ 31 and 29, respectively. The principal chains of amylose forming complexes with MG-C18:1 and MG-C18:0 were $\overline{DP}$ 35–36 and $\overline{DP}$ 33–34, respectively.

Key words: amylose complex, glycerol monooleate, glycerol monostearate, amylose chain-length

The use of fatty-acid monoglyceride to form a complex with starch has been used as a quality improvement agent for anti-firming or food texturing of starch-based foods such as breads and Japanese noodles. The authors have already reported that monoglycerides and lysophospholipids consisting of unsaturated fatty acids could form complexes with amylose and starch in aqueous solutions.^{1,2)}

The number of glucosyl residues of amylose required to form a complex with a saturated fatty acid was estimated with a computer by imaging a state including the fatty acid in amylose as a helical structure,^{3,4)} but the glucosyl residue calculations seemed to be different from the experimental results^{5–8)} obtained using amyloses with different chain lengths.

In this paper, the sizes of amylose required to form complexes with glycerol monostearate or glycerol monooleate were estimated by analyzing chain distributions of the obtained complexes using high-performance anion exchange chromatography with a pulsed amperometric detector (HPAEC-PAD).

MATERIALS AND METHODS

Materials. Glycerol monostearate (MG-C18:0, purity 99%) and glycerol monooleate (MG-C18:1, purity 97%) were purchased from Funakoshi Co., Ltd. (Tokyo, Japan) and Nippon Oil & Fats Co., Ltd. (Tokyo, Japan), respectively. Amylose EX-1 ($\overline{DP}$ 18; crude protein, 0.02%; moisture, 7.90%) was purchased from Hayashibara Biochemical Laboratories (Okayama, Japan). Methanol, dimethyl sulfoxide (DMSO), potassium hydroxide (KOH) and hydrochloric acid (HCl) were purchased as special-grade materials from Wako Pure Chemical Industries Co., Ltd. (Osaka, Japan). Sodium hydroxide (NaOH) and so-

dium acetate (CH_3COONa) were purchased as HPLC-grade materials from Wako Pure Chemical Industries Co., Ltd.

Preparation of 0.2% (w/v) amylose solution. Amylose EX-1 (2.0 g) dispersed in distilled water (100 mL) was mixed with 1 M KOH (100 mL) and then stirred continuously for 60 min at 4°C. After adjusting the pH to 6.0 with 1 M HCl, the amylose solution was diluted with distilled water to 1000 mL.

Preparation of a complex of lipid and amylose. After dissolving MG-C18:0 (50 mg) or MG-C18:1 (50 mg) in methanol (5 mL), both lipid solutions were dispersed in distilled water (45 mL) and stirred continuously for 30 min at 70°C, respectively. After cooling to 60°C, both monoglyceride solutions were mixed with 250 mL of 0.2% (w/v) amylose solution, respectively, and then stirred occasionally for 30 min at 60°C. After cooling, the mixtures were kept at 20°C for 3 h to complete the inclusion complexes, and then centrifuged at $15,000 \times g$ for 20 min at 20°C.

After the supernatant was decanted carefully, the precipitate obtained was resuspended in distilled water (200 mL) at 20°C and then centrifuged ($15,000 \times g$, 20°C, 20 min) in order to rinse the inclusion complex. This process was conducted twice and the pure inclusion complex (A) was lyophilized.

Preparation of amylose contributed to complex formation. The inclusion complex (10 mg) was dissolved in DMSO (1 mL), mixed with methanol (50 mL) and centrifuged at $15,000 \times g$ for 20 min at 10°C. The precipitate (B) obtained was rinsed with methanol and then lyophilized.

Differential scanning calorimetry. Sample A (5 mg) of MG-C18:0 and MG-C18:1 was weighed on a DSC silver pan (70 μL) with distilled water (20 mg) and the pan was hermetically sealed immediately to prevent moisture loss. The endothermic peak was determined by measuring

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with a differential scanning calorimeter (DSC-100, Seiko Instruments Inc., Tokyo, Japan) at a heating rate of 1°C/min. The onset temperature (T_o) and peak temperature (T_p) of sample A of MG-C18:0 and MG-C18:1 were measured to characterize the thermal property of complex.

HPAEC-PAD analysis. Sample B (1.0 mg) was dissolved in 1 M NaOH (100 μ L), diluted with distilled water (300 μ L) and then centrifuged at 3000 rpm at room temperature. The supernatant (50 μ L) was subjected to HPAEC-PAD analysis using a Dionex BioLC system (Dionex Co., Sunnyvale, CA, USA) with a pulsed amperometric detector (Model PAD II) and a CarboPac PA1 column (250 $\times$ 4 mm I.D.). Eluent A was 150 mM NaOH and eluent B was 150 mM NaOH containing 500 mM CH₃COONa. The gradient program was as follows: % of eluent B, 10 at 0 min, 80 at 80 min and 100 at 87 min, respectively.

RESULTS AND DISCUSSION

Preparation of a lipid and amylose complex.

The complexes of Amylose EX-1 (DP 18) with monoglycerides did not precipitate at 60°C, but crystalline precipitate appeared at 20°C. The inclusion complexes (A) obtained with MG-C18:0 and MG-C18:1 were 51.5 and 42.6 mg, respectively. These results indicate that the complex-forming ability of MG-C18:1 toward amylose is somewhat lower than that of MG-C18:0.

Differential scanning calorimetry.

Many reports have been published on the DSC curve of amylose-lipid complex as proof of complex formation.^{1,2,8-10} The endothermic peak on the DSC curve of the inclusion complex (A) with MG-C18:0 was observed at 59.3 (T_o) and 62.0°C (T_p), and the dissolution enthalpy change (ΔH) was 9.6 J/g (Fig. 1 B). The endothermic peak on the curve of the inclusion complex (A) with MG-C18:1

was observed at 61.5 (T_o) and 64.4°C (T_p), and the ΔH was 3.4 J/g (Fig. 1 C). The temperatures of these peaks are clearly different from the temperatures of MGs (Fig. 1 D, E). The endothermic peak on the curve of Amylose EX-1 alone was not observed (Fig. 1 A). From these results, the occurrence of complexes between the monoglycerides and Amylose EX-1 could be confirmed.

HPAEC-PAD analysis.

The chain-length distribution of Amylose EX-1 is shown in Fig. 2, which is in good agreement with the results of Koizumi *et al.*¹¹ The chain-length distributions of amyloses recovered from the inclusion complexes with the monoglycerides were analyzed by HPAEC-PAD (Fig. 3). The difference in chain-length distribution between the MG-C18:0 precipitate and that of MG-C18:1 is shown in Fig. 4.

The amylose chain profiles recovered from complexes are quite different from that of Amylose EX-1. The critical DP of amylose recovered from the complex with MG-C18:0 was estimated to be DP 29, and the principal chain was DP 33–34. The critical DP of amylose recovered from the complex with MG-C18:1 was estimated to be

Fig. 2. HPAEC-PAD analysis of Amylose EX-1.

Fig. 3. HPAEC-PAD analyses of the amyloses in the precipitates formed by complexes using MG-C18:0 (A) and MG-C18:1 (B).

Fig. 1. DSC thermograms of Amylose EX-1, amylose-MG-C18:0 complex, amylose-MG-C18:1 complex, MG-C18:0 and MG-C18:1 with the addition of water (sample, 5 mg; water, 20 mg; heating rate, 1°C/min).

A, Amylose EX-1; B, amylose-MG-C18:0 complex; C, amylose-MG-C18:1 complex; D, MG-C18:0; E, MG-C18:1. T_o , onset temperature; T_p , peak temperature.

Fig. 4. The differences in chain numbers calculated by subtracting the value of MG-C18:0 precipitate from that of MG-C18:1 precipitate.

DP 31, and the principal chain was DP 35–36. From a comparison of the chain-length distribution of the two complexes, it was found to be obvious that the inclusion complex with MG-C18:1 was composed of longer amylose chains than the inclusion complex with MG-C18:0, as shown in Fig. 4. Amylose chains found in the complex with MG-C18:1 are about 2–5 glycosyl residues longer than those found in the complex with MG-C18:0. It is presumed that the helical structure of amylose including oleic acid in MG-C18:1 needs 2–5 more glucose residues because of the bent structure of the double bond in the fatty acid.

As the amylose used in this study was $\overline{\text{DP}} 18$, the content of amylose fractions longer than DP 29 was extremely low. A study on the inclusion complex of many fatty acids should be conducted using longer amylose chains like DP 30.

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オレイン酸モノグリセリドおよびステアリン酸モノグリセリドと複合体を形成するアミロースの特性

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オレイン酸モノグリセリド (MG-C18:1) およびステアリン酸モノグリセリド (MG-C18:0) と平均重合度 18 のアミロースを用いて、水溶液で複合体を調製した。20°C で沈澱した複合体について、そのアミロース鎖長を HPEAC-PAD で分析した。MG-C18:1 および MG-C18:0 の複合体を形成し始めるアミロース鎖長は、それぞれ DP 31, DP 29 であった。また、MG-C18:1 および MG-C18:0 の複合体を構成する主なアミロース鎖長は、それぞれ DP 35–36, DP 33–34 であった。