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Formation of a new ester compound between triglyceride and dicarboxylic acid catalyzed by lipase

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Abstract

We identified a new interesterification reaction between tripalmitin and dicarboxylic acid using lipase. Tripalmitin and dicarboxylic acid were incubated at 70 °C for 72 h with Lipozyme® RM IM. The reaction mixture was analyzed by MALDI TOF-MS and liquid chromatography/mass spectrometry. The products found were a dimer [(α(γ),β-dipalmitoyl)-glyceryl]-γ(α)-(ω-carbooctadecanoyl)-α′(γ′),β′-dipalmitoyl-glycerol and oligomers of glyceride. This dimer was also found in Japan wax, traditionally used as cosmetics.

Keywords: Interesterification; Triglyceride; Dicarboxylic acid; Lipase

1. Introduction

Lipase is an enzyme that catalyzes not only the hydrolysis of the ester bond of triglyceride and other esters, but also the synthesis of an ester from alcohol and carboxylic acid [1–4]. They are also able to modify triglyceride using fatty acid by an interesterification reaction [1,5,6]. In this case, the reaction is carried out in an organic solvent or solvent-free system [1].

In this study, we attempted the interesterification reaction between tripalmitin and eicosanedioic acids instead of fatty acids by lipase to obtain a glyceride linked by dicarboxylic acid ([(α(γ),β-dipalmitoyl)-glyceryl]-γ(α)-(ω-carbooctadecanoyl)-α′(γ′),β′-dipalmitoyl-glycerol) (Scheme 1). A similar compound has been found in the sumac wax known as Japan wax [7–10]. Due to its viscoductility, Japan wax is often used in cosmetics and similar products.

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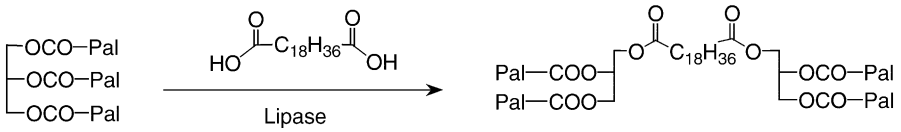
2. Experimental procedure

2.1. Chemicals

The following chemicals were used as substrates: tripalmitin, 1,20-eicosanedioic acid and sebacic acid (Tokyo Kasei Kogyo Co. Ltd., Japan). Lipozyme® RM IM (RM IM) (immobilized lipases) was gifts from Novozymes Japan Ltd. The 2,5-dihydroxybenzoic acid used for the matrix and HPLC-grade chloroform for gel permeation chromatography (GPC) and liquid chromatography/mass spectrometry (LC/MS) were obtained from Wako Pure Chemicals Co., Japan. The chloroform was filtered through Milicup™ (Millipore Co., Bedford, MA, USA) before use. Japan wax was purchased from Araki Seirou Co., Japan.

2.2. Analysis of Japan wax

One gram of Japan wax was dissolved in 40 ml of acetone at 60 °C, and after cooling the solution in a refrigerator, filtered through a filter paper under vacuum. The residue



Scheme 1. Interesterification reaction between triglyceride and dicarboxylic acid.

obtained by the filtration was named Fraction 1, the filtrate Fraction 2. Tripalmitin and products in Fraction 1 were analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF-MS), GPC and LC/MS.

2.3. Reaction conditions in solvent-free system

In a solvent-free system, 1.0 g of tripalmitin (1.24 mmol, melting point around 67 °C) was melted at 70 °C in a test tube, to which were added 0.21 g of eicosanedioic acid (0.62 mmol) and 1.0 g of lipase RM IM (150 IUN/g, One Interesterification Unit Novo is defined as 0.01 w/w% converted tristearin/min/g enzyme product at the following batch interesterification conditions: substrate (fully hydrogenated soybean oil/soybean oil; 27/73 w/w%, temperature; 70 °C, no co-solvents). The reaction mixture was incubated at 70 °C for 72 h with magnetic stirring. At the end of the reaction, chloroform was added to the reaction mixture and insoluble lipase was removed by filtration through a filter paper under vacuum. After evaporation of chloroform, the residue was dissolved in acetone and the solution was cooled in a refrigerator. It was filtrated through filter paper. The residue was named Fraction 1 and the filtrate Fraction 2, as shown in Fig. 1. The molecular weight of Fractions 1 and 2 were analyzed by MALDI TOF-MS.

2.4. MALDI TOF-MS

Five milligrams of the sample was dissolved in 100 μl tetrahydrofuran and 50 mg 2,5-dihydroxybenzoic acid as the matrix was dissolved in tetrahydrofuran 200 μl. Sample solution (5 μl) and matrix solution (20 μl) were mixed and analyzed. A Voyager-DE PRO mass spectrometer (PerSeptive Biosystems, U.S.A.) was used. Analytical conditions were as follows: accelerating voltage 20,000 V, guide wire voltage 0.050%, mode reflector, low mass gate *m/z* 400.

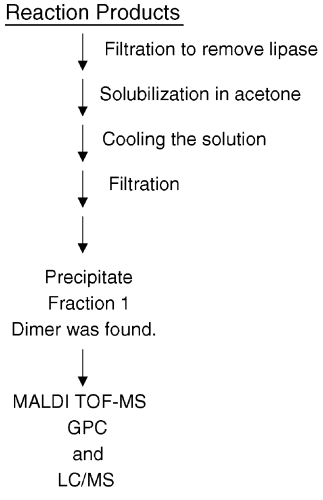


Fig. 1. Harvest scheme of target compound from reaction mixture.

2.5. Gel permeation chromatography

For GPC, a degasser, a pump, a mixer, a column and a reflective index detector were used as a system (Jasco Co. Ltd., Japan). A Shodex Asahipak GF-310 HQ column and a Shodex Asahipak GF-1G 7B guard column (Showa Denko Co. Ltd., Japan) were used. A 10 μl sample (10 mg/ml) was injected into the equipment for analysis. The column was kept at 37 °C, and chloroform was used as an eluent at a flow rate of 0.6 ml/min. System control and data evaluations were done using BORWIN software (Jasco Co. Ltd.).

2.6. Liquid chromatography/mass spectrometry

An Agilent 1100 LC/MSD was used for the GPC system, and the specimen was analyzed as described above. Mass spectrometry was performed fundamentally according to the method of Mcintyre [11]: scan range 100–2200 *m/z*, drying gas flow 5 ml/min and fragmenter 0.8 V.

3. Results and discussion

3.1. MALDI TOF-MS analyses of the products

We chose a solvent-free system instead of using organic solvents in the reaction to eliminate the inhibitory effect of an organic solvent on the enzyme [1]. Eicosanedioic acid was used as an acyl donor to interesterify palmitic acid in tripalmitin using lipase RM IM. Fraction 1 obtained by precipitation in acetone (0.78 g) was subjected to MALDI TOF-MS analyses. Tripalmitin was detected at *m/z* 830, α(γ)-(ω-carbooctadecanoyl)-γ(α),β-dipalmitoyl-glycerol at *m/z* 916, [(α(γ),β-dipalmitoyl)-glyceryl]-γ(α)-(ω-carbooctadecanoyl)-α′(γ′),β′-dipalmitoyl-glycerol (dimer) at *m/z* 1467 and a trimer of three tripalmitins linked by two eicosanedioic acids at *m/z* 2104 (Fig. 2). The peak, *m/z* 551 was shown to be a fragment ion of tripalmitin according to Reid Asbury et al. [12], and the peaks of *m/z* 1187 and 1188 were similarly considered to be fragment ions of the dimer. Dipalmitin (*m/z* 591) and the compound of monopalmitin and eicosanedioic acid (*m/z* 677) were in Fraction 2 (data not shown).

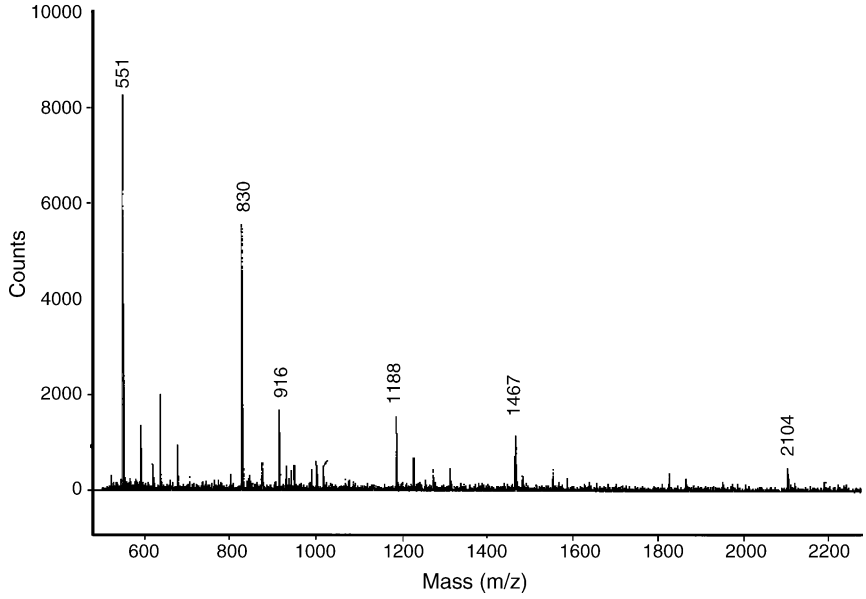


Fig. 2. MALDI TOF-MS of Fraction 1.

3.2. Comparison between the products and Japan wax using LC/MS

The molecular weights of components in Fraction 1, which passed through a GPC column, were determined by LC/MS (Fig. 3). (A) shows the total ion chromatogram (TIC) and (B–E) show extracted ion chromatograms (EIC). The peak of *m*/*z* 2104 is considered to be a trimer, *m*/*z* 1467 a dimer, *m*/*z* 830 tripalmitin and *m*/*z* 916 α(γ)-(ω-carbooctadecanoyl)-γ(α),β-dipalmitoyl-glycerol. Thus, the various products were isolated by using a GPC column. The mass spectrum of (C) by atmospheric pressure chemical ionization (APCI) revealed signals at *m*/*z* 551.2, 875.5 and 1188.3. Similarly, signals at *m*/*z* 551.3, 875.3 and 1187.5 were detected in terms of Japan wax. The relative intensities of the mass fragments in these spectra were almost the same. These results of LC/MS analysis using APCI suggested the dimer ([(α(γ),β-dipalmitoyl)-glyceryl]-γ(α)-(ω-carbooctadecanoyl)-α′(γ′),β′-dipalmitoyl-glycerol) synthesized by lipase RM IM and that Japan wax contained the same compound. However, we could not determine the position of dicarboxylic acid combined with glyceride. Considering that the lipase used was of the 1,3-regiospecific type [13], the interesterified product was suggested to [(α(γ),β-dipalmitoyl)-glyceryl]-γ(α)-(ω-carbooctadecanoyl)-α′(γ′),β′-dipalmitoyl-glycerol.

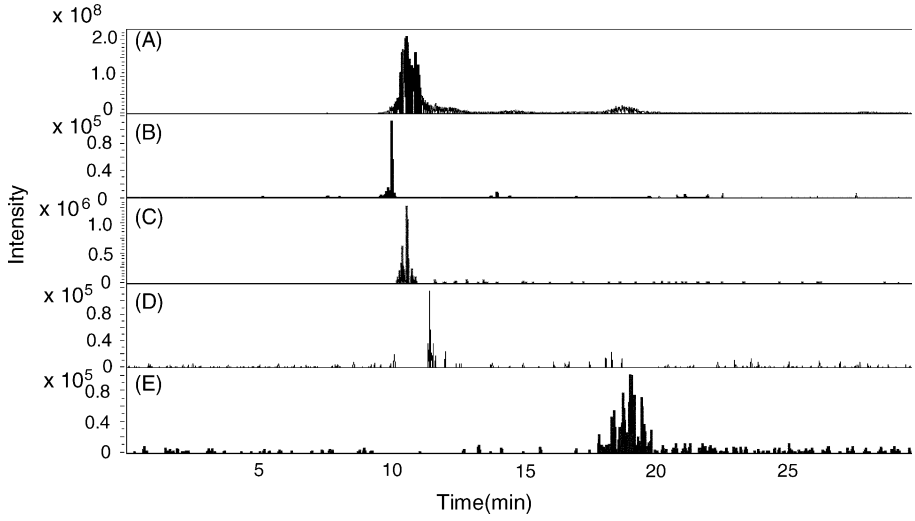
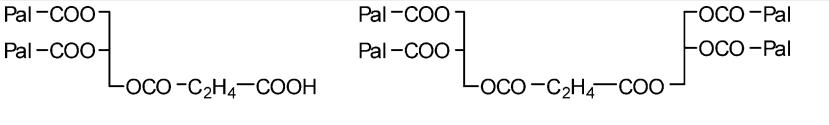
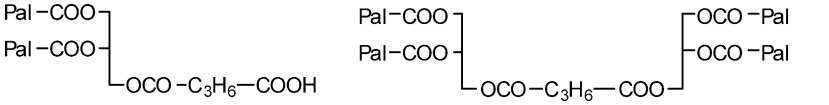
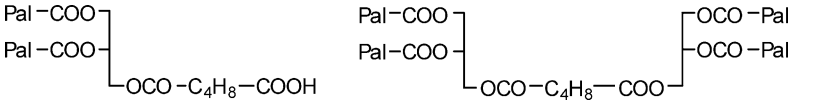
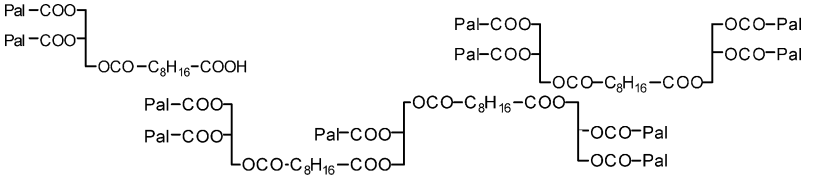
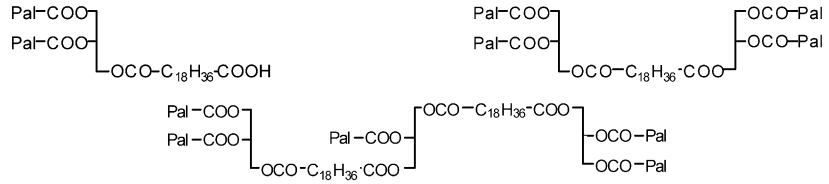


Fig. 3. Mass chromatography of Fraction 1: (A) total ion chromatogram (TIC), (B) mass chromatogram at *m*/*z* 2104, (C) mass chromatogram at *m*/*z* 1467; (D) mass chromatogram at *m*/*z* 830 and (E) mass chromatogram at *m*/*z* 916.

Table 1
Products of the interesterification reaction between tripalmitin and dicarboxylic acid having various carbon chain length

Dicarboxylic acid	Detected products
Succinic acid	
Glutaric acid	
Adipic acid	
Sebacic acid	
1,14-tetradecanedioic acid	

3.3. Quantitative analysis of the products

Tachibana et al. [10] reported the production of [(α″,β′-dipalmitoyl)-glyceryl]-α-(ω-carbooctadecanoyl)-α′, β-dipalmitoyl-glycerol from tripalmitin and α(γ)-(ω-carbooctadecanoyl)-γ(α),β-dipalmitoyl-glycerol, chemically synthesized from glycerol, as substrates. In our experiment, we succeeded in the enzymatic synthesis of the

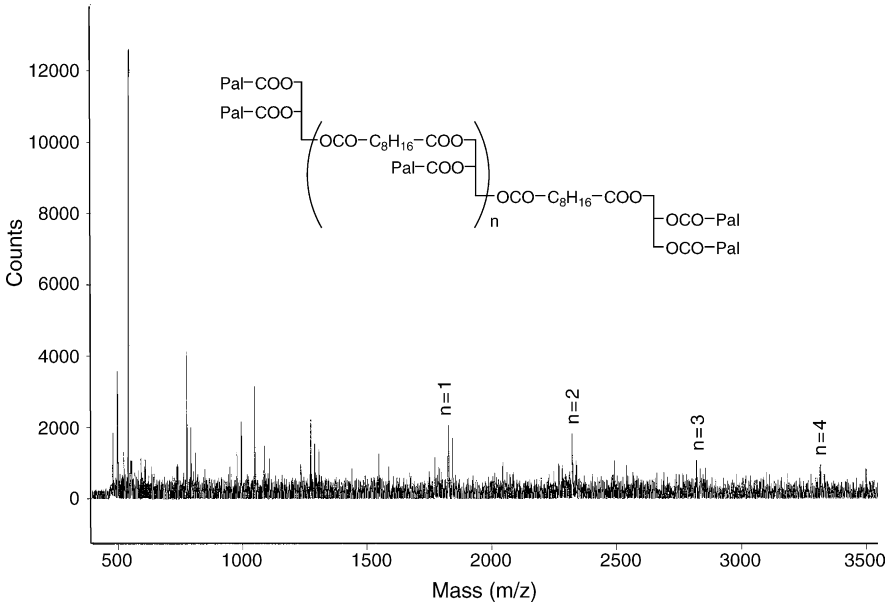


Fig. 4. Oligomer interesterification reaction between tripalmitin and sebacic acid by RM IM.

same product (yield against consumed tripalmitin, 18%) in a simple reaction using tripalmitin and eicosanedioic acid. The dimer content of this product (0.11 mmol/1 g of sample) is richer than that that in native Japan wax (0.04 mmol/1 g of sample). The product found in this study is expected to be utilized as a substitute for Japan wax. Succinic acid, glutaric acid, adipic acid, sebacic acid and 1,14-tetradecanedioic acid gave similar products in the interesterification reaction. The products detected by mass spectrum are shown in Table 1. This is the first report that deals with the interesterification reaction between tripalmitin and dicarboxylic acid (shown as above) using lipase. The interesterified products were not only a dimer [10] but also oligomers of diglyceride. Moreover, the molecular weight analyses of the product (tripalmitin and sebacic acid by lipase RM IM) revealed various oligomers (trimer-hexamers), as shown in Fig. 4.

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