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Thermoplasticization of euglenoid β-1,3-glucans by mixed esterification

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ABSTRACT

We experimentally demonstrated that paramylon, a storage polysaccharide of *Euglena gracilis*, is efficiently thermoplasticized by adding acyl groups that differ in alkyl chain length. Glass transition temperature of mixed paramylon esters was higher than those of plant-based polylactic acid (PLA), poly 11-aminoundecanoic acid (PA11), and petroleum-based acrylonitrile-butadiene-styrene (ABS) resin and was comparable to that of cellulose acetate stearate (CAS). Their thermoplasticity was equivalent to or higher than those of these reference plastics. The bending strength and bending elastic modulus of injection molded test specimens made from mixed paramylon esters were comparable to those of the reference plastics. While their impact strength was lower than that of specimens made from ABS resin and CAS, it was comparable to those of PLA and PA11. Euglenoid β-1,3-glucans are thus a potential component of thermoplastic materials.

1. Introduction

The use of microalgae to produce useful chemicals has recently attracted attention because they can transform carbon dioxide and water into carbohydrates by photosynthesis (Chisti, 2007). Attention has particularly focused on production of biofuels as part of the effort to reduce dependence on carbon-based fuels (Banerjee, Sharma, Chisti, & Banerjee, 2002; Kaya et al., 2011; Mata, Martins, & Caetano, 2010; Tucci, Vacula, Krajcovic, Proksch, & Martin, 2010; Yamane et al., 2013). By contrast, less attention has been directed at the use of microalgae to create other useful difficult-to-synthesize carbohydrates. Our efforts in this area have focused on *Euglena*-derived storage polysaccharide (β-1,3-glucan), which is generally referred to as paramylon (Shibakami, Sohma, & Hayashi, 2012; Shibakami, Tsubouchi, Nakamura, & Hayashi, 2013).

Since paramylon has unique helical structures due to its β-1,3-bonds, it is likely that materials made from this polysaccharide will exhibit intriguing thermal and mechanical properties that differ from those of materials made from other polysaccharides. One physical property that is useful for preparing materials of various shapes is thermoplasticity. Paramylon, however, does not inherently have this property. It has been reported that mixed cellulose esters having at least two types of substituents exhibit thermoplasticity (Peydecastaing, Vaca-Garcia, & Borredon, 2011; Teramoto, Yoshioka, Shiraishi, & Nishio, 2002). Wax esters, i.e., esters of long-chain fatty acid and higher alcohol, are another major euglenoid product in addition to paramylon. Of particular interest is that hydrolysis of wax esters releases the fatty acid and higher alcohol. We hypothesized that if wax ester-derived fatty acids are introduced into paramylon, we will likely obtain thermoplastic mixed paramylon esters with a *high euglenoid constituent ratio*. Toward this goal, we have started a program aimed at synthesizing mixed paramylon esters containing long-chain fatty acids.

Here we report the creation of thermoplastic paramylon derivatives. To the best of our knowledge, this is the first creation of *Euglena*-based thermoplastics despite the long study of *Euglena* since the establishment of genus *Euglena* by Ehrenberg in 1830 (Gojdics, 1953). The primary objective of the work described here was twofold: (i) to establish a mix-esterification method that provides thermoplastic paramylon derivatives and (ii) to investigate the relationship between the chemical structures of the derivatives and their thermal and mechanical properties.

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2. Experimental

2.1. General methods

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AVANCE 500 spectrometer. Quantitative ^{13}C NMR spectra were obtained by means of the inverse gated decoupling method. Fourier transform infrared (FT-IR) spectra were recorded using a JASCO FT/IR-480ST spectrophotometer equipped with an attenuated total reflectance accessory (ATR Pro 400-S, ZnSe prism, JASCO) with a resolution of 4 cm^{-1}. Melting behavior was observed using a Yanako MP-500D melting-point apparatus. Elemental analyses were performed using a CE Instruments EA1110 elemental analyzer.

All chemicals and reagents except for the paramylon used for synthesizing the paramylon derivatives are commercially available and were used without further purification. N,N-dimethylacetamide (DMAc), lithium chloride (LiCl), and triethylamine (NEt_3) were purchased from Sigma–Aldrich, Kishida Chemical, and Junsei Chemical, respectively. Myristoyl, palmitoyl, and stearoyl chloride were purchased from Wako Pure Chemical Industries, Tokyo Chemical Industry, and Kanto Chemical, respectively. Methanol and chloroform were obtained from Kishida Chemical and Junsei Chemical, respectively. Acetic anhydride and pyridine were obtained from Tokyo Chemical Industry and Sigma–Aldrich, respectively. Polylactic acid (PLA, TE-4000), poly-11-aminoundecanoic acid (PA11, Rilsan BMFO), and acrylonitrile-butadiene-styrene (ABS) resin (GA-701) were purchased from Unitika, Arkema Japan, and Nippon A&L Co, respectively. Paramylon particles were obtained from *Euglena gracilis* in accordance with a previously reported method (Shibakami et al., 2012). The degree of substitution (DS) values, which are the average number of functional groups attached to a glucose unit, were determined by comparing the integral values of the methyl protons of the long alkyl chains and acetyl groups with those of the glucosidic protons in the ^{1}H NMR spectrum; DS_{lac} and DS_{ace} in the text represent the DS of the long alkyl chains (myristoyl, palmitoyl, and stearoyl) and acetyl groups, respectively.

2.2. Synthesis of mixed paramylon esters

2.2.1. Paramylon acetate myristate (DS_{lac} 0.28/DS_{ace} 2.49) (**1a**)

To a homogeneous solution of paramylon (10.0 g, 61.7 mmol) in DMAc (0.5 L)/LiCl (8.0 g, 188.7 mmol) prepared by heating at ~110 °C for 0.5 h were added dropwise 7.8 mL (56.0 mmol) of NEt_3 and 0.5 L of a DMAc solution containing myristoyl chloride (8.4 mL, 30.9 mmol) at room temperature. This mixture was heated at 110 °C under a nitrogen atmosphere for 3 h. Methanol (2.0 L) was then added to precipitate a solid. This solid was washed with a mixture of methanol and chloroform (0.9 L, 1/2 (v/v)). Air drying overnight and subsequent vacuum drying at 70 °C for 2 h and 105 °C for 4 h produced paramylon myristate as a solid (13.0 g, 58.8 mmol).

To a homogeneous solution containing DMAc (1.5 L), LiCl (6.6 g, 155.7 mmol), and paramylon myristate (12.5 g, 56.5 mmol), which was prepared by heating at ~110 °C for 0.5 h, were added pyridine (168 mL, 2.09 mol) and acetic anhydride (240 mL, 4.89 mol) at 70 °C. The mixture was heated at 70 °C for 6 h and then left at ambient temperature for 17 h under a nitrogen atmosphere. Addition of water (3.0 L) to the mixture produced a solid. This solid was washed with water (1.6 L) and methanol (0.3 L). Air drying overnight and subsequent vacuum drying at 80 °C for 1 h and 105 °C for 4 h produced paramylon acetate myristate (**1a**) as a solid (15.1 g, 46.4 mmol, yield 75.2%). Successful preparation was confirmed by FT-IR and ^{1}H and ^{13}C NMR measurements. ^{1}H NMR ($CDCl_3$) δ 4.99–4.75 (m), 4.47–4.20 (m), 4.08–3.96 (m), 3.79–3.57 (m), 2.37–2.21 (m), 2.12 (s), 2.06 (s), 2.00 (s), 1.57 (m), 1.26 (s), 0.88 (t, J = 6.9). ^{13}C NMR ($CDCl_3$) δ 173.4, 170.5, 169.0, 168.8, 100.7, 78.4, 72.7, 71.8, 68.1, 62.0, 33.9, 31.9, 29.6, 29.5, 29.3, 29.2, 29.1, 24.6, 22.6, 20.8, 20.7, 20.4, 14.1. FT-IR (cm^{-1}) 2875, 2807, 1740, 1365, 1212, 1033, 886.

2.2.2. Paramylon acetate myristate (DS_{lac} 0.61/DS_{ace} 2.23) (**1b**)

By using procedures similar to those described for the preparation of **1a**, a 52.7% yield of **1b** (12.5 g, 32.5 mmol) was obtained; ^{1}H NMR ($CDCl_3$) δ 4.99–4.75 (m), 4.47–4.20 (m), 4.08–3.96 (m), 3.79–3.57 (m), 2.37–2.21 (m), 2.11 (s), 2.07 (s), 1.99 (s), 1.58 (m), 1.25 (s), 0.87 (t, J = 6.9). ^{13}C NMR ($CDCl_3$) δ 173.4, 170.5, 169.0, 168.8, 100.7, 78.4, 72.7, 71.8, 68.1, 62.0, 33.9, 31.9, 29.6, 29.5, 29.3, 29.2, 29.1, 24.6, 22.6, 20.9, 20.7, 20.5, 14.1. FT-IR (cm^{-1}) 2918, 2849, 1740, 1364, 1210, 1029, 888.

2.2.3. Paramylon acetate palmitate (DS_{lac} 0.29/DS_{ace} 2.41) (**1c**)

By using procedures similar to those described for the preparation of **1a**, a 71.6% yield of **1c** (14.7 g, 44.2 mmol) was obtained; ^{1}H NMR ($CDCl_3$) δ 4.99–4.75 (m), 4.47–4.20 (m), 4.08–3.96 (m), 3.79–3.57 (m), 2.37–2.21 (m), 2.11 (s), 2.06 (s), 2.00 (s), 1.56 (m), 1.26 (s), 0.88 (t, J = 6.9). ^{13}C NMR ($CDCl_3$) δ 173.4, 170.5, 169.0, 100.7, 78.4, 72.7, 71.8, 68.1, 62.0, 33.9, 31.9, 29.7, 29.5, 29.3, 29.1, 24.6, 22.6, 20.9, 20.7, 20.4, 14.1. FT-IR (cm^{-1}) 2911, 2846, 1741, 1365, 1213, 1032, 887.

2.2.4. Paramylon acetate palmitate (DS_{lac} 0.50/DS_{ace} 2.02) (**1d**)

By using procedures similar to those described for the preparation of **1a**, a 59.8% yield of **1d** (13.5 g, 36.9 mmol) was obtained; ^{1}H NMR ($CDCl_3$) δ 4.99–4.75(m), 4.47–4.20(m), 4.08–3.96(m), 3.79–3.57 (m), 2.37–2.21 (m), 2.11 (s), 2.05 (s), 1.98 (s), 1.58 (m), 1.25 (s), 0.87 (t, J = 6.9). ^{13}C NMR ($CDCl_3$) δ 173.4, 170.5, 169.0, 168.8, 100.7, 78.4, 72.7, 71.8, 68.2, 62.0, 33.9, 31.9, 29.7, 29.5, 29.3, 29.2, 29.1, 24.7, 22.7, 20.8, 20.7, 20.5, 20.4, 14.1. FT-IR (cm^{-1}) 2914, 2849, 1747, 1363, 1213, 1032, 890.

2.2.5. Paramylon acetate stearate (DS_{lac} 0.31/DS_{ace} 2.33) (**1e**)

By using procedures similar to those described for the preparation of **1a**, a 82.8% yield of **1e** (17.5 g, 51.1 mmol) was obtained; ^{1}H NMR ($CDCl_3$) δ 4.99–4.75 (m), 4.47–4.20 (m), 4.08–3.96 (m), 3.79–3.57 (m), 2.37–2.21 (m), 2.12 (s), 2.06 (s), 2.00 (s), 1.55 (m), 1.26 (s), 0.88 (t, J = 6.9). ^{13}C NMR ($CDCl_3$) δ 173.4, 170.5, 169.0, 100.7, 78.4, 72.6, 71.8, 68.1, 62.1, 33.9, 31.9, 29.7, 29.5, 29.3, 29.1, 24.6, 22.6, 20.8, 20.7, 20.4, 14.1. FT-IR (cm^{-1}) 2920, 2847, 1740, 1365, 1212, 1031, 889.

2.2.6. Paramylon acetate stearate (DS_{lac} 0.46/DS_{ace} 2.07) (**1f**)

By using procedures similar to those described for the preparation of **1a**, a 48.8% yield of **1f** (11.2 g, 30.1 mmol) was obtained; ^{1}H NMR ($CDCl_3$) δ 4.99–4.75 (m), 4.47–4.20 (m), 4.08–3.96 (m), 3.79–3.57 (m), 2.37–2.21 (m), 2.12 (s), 2.08 (s), 2.00 (s), 1.58 (m), 1.25 (s), 0.87 (t, J = 6.9). ^{13}C NMR ($CDCl_3$) δ 173.4, 170.6, 169.1, 100.7, 78.5, 72.7, 71.8, 68.2, 62.0, 33.9, 31.9, 29.7, 29.5, 29.4, 29.2, 24.7, 22.7, 20.9, 20.7, 20.5, 14.1. FT-IR (cm^{-1}) 2920, 2849, 1740, 1366, 1209, 1030, 890.

2.2.7. Paramylon dimyristate (DS_{lac} 2.08) (**2**)

To a homogeneous solution of paramylon (100 mg, 0.62 mmol) in DMAc (5.0 mL)/LiCl (79 mg, 1.86 mmol) prepared by heating at ~110 °C for 0.5 h were added dropwise 1.3 mL (9.34 mmol) of NEt_3 and 15 mL of a DMAc solution containing myristoyl chloride (1.3 mL, 4.79 mmol) at room temperature. This mixture was heated at 120 °C under a nitrogen atmosphere for 3 h. Methanol (40 mL) was then added to precipitate a solid. This solid was washed with a mixture of methanol and chloroform (30 mL, 2/1 (v/v)). Air drying overnight and subsequent vacuum drying at 90 °C for 2 h produced paramylon dimyristate (**2**) as a solid (390 mg, 0.65 mmol,