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Synthesis and characterization of pullulan alkyl esters

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ABSTRACT

Fully substituted pullulan (PL) esters with carbon number of acyl group (n) of 2–14 were prepared in carboxylic acid/trifluoroacetic anhydride (TFAA) system. Thermal properties of PL esters were analyzed by differential scanning calorimetry (DSC) and thermogravimetric analyses (TGA). The decomposition temperatures of the PL esters were higher than that of neat PL. DSC measurements revealed that the PL esters did not exhibit melting peaks, indicating that PL esters were amorphous. The glass transition temperatures (T_gs) of PL esters increased from 35 °C for PL myristate (PLMy, $n = 14$) to 174 °C for PL acetate (PLAc, $n = 2$) as the chain length decreased. WAXD diffractogram of the PL esters showed the reflection at small-angle region, indicating alignment of the PL main-chain caused by side-chains which are extended perpendicular to the main-chain. Colorless and transparent PL ester films were obtained by solvent casting and melt-pressing method. DMA analyses indicated a β-relaxation of alkyl side-chains below room temperature. Tensile strength of the PL ester films decreased with increase in alkyl carbons from 23.4 MPa for PLAc to 1.4 MPa for PLMy. The elongation at break of the PL esters increased with increase in alkyl carbons from 8% for PLAc to 1090% for PLMy. The PL esters exhibited extremely high elongation at break. PL esters could be electrospun into nanofibers.

1. Introduction

Pullulan (PL) is a linear polysaccharide extracellularly produced by strains of fungus *Aureobasidium pullulans*, and is consisting of α-(1 → 6) linked maltotriose units, which is α(1 → 4) linked three glucose units [1,2]. PL is regarded as the one of the biodegradable and renewable materials obtained from the nature. PL has valuable properties such as water-soluble, non-toxic, edible, and film-forming abilities, and is widely used in food or biomedical field [2–5].

Although many studies on the chemical modification of PL, such as succinylation [6], modification with isocyanates [7] or cholesteroyl group [8] or sulfation [9], have been reported, the possibility of PL as plastic material has not been fully explored. It is difficult to process PL by conventional melt processing technique to obtain plastic material, because it degrades thermally at around 250 °C without a clear appearance of glass transition temperature (T_g) and melting temperature (T_m) on heating [7]. Therefore, preparation of PL derivatives, such as PL acetate [10–12] or PL alkyl propyl and butyl ethers [13], have been attempted with the purpose of their application as a plastic material.

Generally, the esterification of polysaccharides including PL, reduce their hydrogen bond network, and alter their properties, for example, to water-insoluble, soluble in organic solvents or melt-processible. For example, cellulose esters, such as cellulose acetate propionate and cellulose acetate butyrate, exhibit thermo-processibility and convertible to films or fibers which have been widely used in industry [14]. There have been substantial literatures on preparation of alkyl esters of polysaccharides, such as

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cellulose [15–17], starch [18–20], chitin [21–23]. Our research group has reported the preparation of alkyl esters of various non-cellulose polysaccharides with the aim of their application as plastic material. Fully substituted esters of xylan [24,25], glucomannan [26–28] or curdlan [29], have been prepared and their possibility as plastic materials has been demonstrated. Preparation of fully substituted PL esters with longer alkyl chains will be effective approach to utilize PL as thermoplastic materials in industry, as well as other polysaccharide esters mentioned above.

In the present study, we present the syntheses of fully substituted PL alkyl esters with different side-chain lengths in carboxylic acid (C2 to C14)/trifluoroacetic anhydride (TFAA) system. The chemical structure of the obtained compounds, their molecular weights, film-forming ability, thermal and mechanical properties were investigated and presented.

2. Experimental

2.1. Materials

Pullulan was purchased from the Tokyo Chemical Industry Co. (Tokyo, Japan). Trifluoroacetic anhydride (TFAA), acetic acid, propionic acid, butyric acid, valeric acid, hexanoic acid, octanoic acid, decanoic acid, lauric acid, and mylistic acid were purchased from Wako Pure Chemicals (Tokyo, Japan), and used without further purification.

2.2. Preparation of PL esters

A representative procedure, for PL acetate, is as follows. A premixed solution of TFAA (10 ml) and acetic acid (10 ml), which had been stirred at 50 °C for 20 min, was immediately added to the freeze-dried PL (1 g) in a flask. The solution was stirred at 50 °C for 1.0 h under nitrogen. After cooling to room temperature, the solution was poured into ethanol (1.0 l). The precipitate was filtered, washed with ethanol, dissolved in chloroform, and reprecipitated in ethanol, before finally being filtered, washed with ethanol, and dried *in vacuo* to give a solid compound, PL acetate (PLAc, $n = 2$) (1.57 g, 88% yield). For other PL esters, appropriate carboxylic acids (10 mL or 10 g; $n = 3$–14) were used instead of acetic acid. The yields (%) of the obtained PL esters were as follows: PL propionate (PLPr, $n = 3$) (1.85 g, 91%), PL butylate (PLBu, $n = 4$) (1.85 g, 81%), PL valerate (PLVa, $n = 5$) (2.03 g, 79%), PL hexanoate (PLHe, $n = 6$) (2.20 g, 78%), PL octanoate (PLOc, $n = 8$) (2.52 g, 78%), PL decanoate (PLDe, $n = 10$) (2.99 g, 76%), PL laurate (PLLa, $n = 12$) (3.11 g, 71%), PL myristate (PLMy, $n = 14$) (3.07 g, 75%). The degree of substitution (DS) of pullulan esters were calculated from the ratio of the integrated area of the methyl protons of the acyl group to the ring protons of glucose, as follows: $\mathrm{DS} = ([\mathrm{CH_3}]/3)/([\text{ring-H}]/7)$.

2.3. Nuclear magnetic resonance (NMR) measurements

^{1}H NMR spectra were recorded with a JEOL JNM-A500 FT NMR (500 MHz) spectrometer, using tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) and coupling constants (J) were reported in ppm and Hz, respectively.

2.4. Gel permeation chromatography (GPC) measurements

Number- and weight-average molecular weights (M_n and M_w) and polydispersity index values (M_w/M_n) were estimated by GPC (CBM-20A, DGU-20A3, LC-6AD, SIL-20ACHT, CTO-20A, RID-10A, Shimadzu) in chloroform at 40 °C. Shodex columns (K-806M, K-802) were used, and the flow rate was 0.8 ml/min. A calibration curve was obtained using polystyrene (PS) standards (Shodex).

2.5. Thermogravimetric (TGA) analysis

TGA was carried out using a Thermo Plus TG 8120 (Rigaku) instrument under a nitrogen atmosphere. Thermograms were acquired between 30 and 450 °C at a heating rate of 20 °C/min.

2.6. Differential scanning calorimetry (DSC) measurements

Differential scanning calorimetry (DSC) thermograms were recorded on a DSC8500 (Perkin–Elmer) under a nitrogen atmosphere. The measurements were made with a 2 mg sample of PL ester powder on a DSC pan. The samples were first heated from −70 °C to 250 °C at the rate of 200 °C/min, cooled to −70 °C at the rate of 100 °C/min and then scanned with heating from −70 °C to 250 °C at the rate of 200 °C/min.

2.7. Wide-angle X-ray diffraction analysis

Wide-angle X-ray diffraction (WAXD) was investigated by a Rigaku RINT 2000 system operating at 40 kV and 200 mA. Measurements were performed with a Bragg–Bretano type $2\theta/\theta$ goniometer in reflection mode. Ni-filtered Cu Kα radiation ($\lambda = 0.15418$ nm) was collimated in a 1/2° divergence slit, 1/6° scatter slit and 0.15 mm receiving slit. Scans were performed twice in the 2θ range 2–40° with a scan rate of 0.5°/min and a step size of 0.1°. PL ester powder was used as a sample.

2.8. Dynamic mechanical analysis (DMA)

DMA measurements were performed with a DVA-200S analyzer (IT Measurement Control, Osaka, Japan). Temperature scans at a frequency of 1 Hz were carried out in the range −150 to 200 °C at a heating rate of 5 °C/min under a nitrogen atmosphere. The initial gauge length was 10 mm. The specimens (18×5 mm^2) were prepared from melt-pressed film.

2.9. Tensile tests

Tensile tests were carried out at room temperature using an EZ-test machine (Shimadzu, Japan). The crosshead speed was 10 mm/min, and the initial gauge length was 10 mm. Five specimens (15×5 mm^2) of the PL ester