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Thickening and water-absorbing agent made from euglenoid polysaccharide

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ABSTRACT

Paramylon, a storage polysaccharide of *Euglena gracilis*, is a linear β-1,3-glucan with a weight-average molecular weight of ~2.0×10^5. Sequential long-chain acylation and succinylation of paramylon yielded amphiphilic paramylon acylate succinates. Owing to their amphiphilicity, these paramylon derivatives showed higher viscosity than paramylon succinate when dispersed in an aqueous solution. Examination of the viscosity of aqueous solutions containing paramylon acylate succinates differing in chain length and degree of substitution of long-chain acyl groups (DS_{lca}) revealed that the longer the acyl chain and the higher the DS_{lca}, the higher the viscosity of the aqueous solution. Solution casting yielded transparent and mechanically tough films from paramylon acylate succinates. These films had high water absorbability, up to ~1000 times their weight.

1. Introduction

Thickening is a typical physical phenomenon exhibited by polysaccharides. Polysaccharide-based thickening agents fall into two categories: intact polysaccharides and chemically modified ones. The former includes guar gum (Thombare, Jha, Mishra, & Siddiqui, 2016), carrageenan (Zia et al., 2016), xanthan gum (Djekic et al., 2016), and succinoglycan (Harada, Misaki, & Saito, 1968; Kaneda, Kobayashi, Miyazawa, & Yanaki, 2002). The latter contains carboxymethyl cellulose (Ragheb, Nassar, Abd El-Thalouth, Ibrahim, & Shahin, 2012), hydroxypropyl methylcellulose (Ji et al., 2010), and other functional group-containing polysaccharides (Falkeborg, Paitaid, Shu, Pérez, & Guo, 2015; Sun, Sun, Wei, Liu, & Zhang, 2007; Szopinski & Luinstra, 2016; Zhao & Chen, 2007; Zhao, Khin, Chen, & Chen, 2005).

In a previous paper, my group synthesized paramylon succinate from paramylon, a euglenoid β-1,3-glucan (Shibakami, Gen Tsubouchi, Makoto Nakamura, & Masahiro Hayashi, 2013). This chemically modified polysaccharide structurally mimics succinoglycan in terms of carrying a succinate group (Fig. S1). Although it was not mentioned in the previous paper, we found that an aqueous solution containing paramylon succinate was highly viscous. The structural and viscosity mimicry between paramylon succinate and succinoglycan gave me the idea that paramylon succinate is a promising starting material for creating a highly efficient thickening agent. Addition of a long-chain acyl group is a promising means for enhancing the thickening ability of water-soluble, cellulose-based polymers. This enhancement stems from the increase in the number of junction points due to the association between the hydrophobic alkyl groups (Akiyama et al., 2005; Akiyama, Kashimoto, Hotta, & Kitsuki, 2006; Akiyama et al., 2007; Tanaka, Meadows, Phillips, & Williams, 1990; Tanaka, Meadows, Williams, & Phillips, 1992; Tanaka, Williams, Meadows, & Phillips, 1992; Um, Poptoshev, & Pugh, 1997). I hypothesized that this design concept should also be effective for paramylon succinate-based polymers. To investigate this possibility, I chemically modified paramylon succinate by adding a long-chain acyl group.

In this report, I describe the relationship between the chemical structure of paramylon acylate succinates and their thickening ability. I also discuss the water absorbability of solution cast films made from the paramylon derivatives.

2. Experimental

2.1. General methods

^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker AVANCE 500 spectrometer (500 MHz). 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

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resolution of 4 cm^{-1}. The degree of substitution of the long-chain acyl group (DS_{lca}) and the acetyl group (DS_{ace}), which is the average number of functional groups attached to a glucose unit, was determined from the ^1H NMR spectrum by comparing the integral values of the methyl protons of the long-chain acyl groups with those of the glucosic and acetyl protons of polysaccharide mixed esters. The degree of substitution of succinate group (DS_{suc}), which is the average number of a succinyl group attached to the glucose unit, was determined using the neutral titration method. The procedure was as follows. First, ~50 mg of succinylated polysaccharide was dispersed in 30 mL of Milli-Q water, and the mixture was mechanically stirred at ambient temperature until homogeneous. Then, 3.0 mL of a 1.0 mol/L NaOH aqueous solution was added to the homogeneous solution, followed by mechanical stirring for ~3 h. The resulting solution was titrated with a 0.10 mol/L HCl aqueous solution using phenolphthalein (two drops of a 1.0-wt% ethanol solution) as an indicator. The equations used for the calculation were as follows.

$$2 \times x + y = 1.0(\text{mol/L}) \times 3.0(\text{mL}) - 0.10(\text{mol/L}) \times a \quad (1)$$

$$y = DS_{lca} \times z \quad (2)$$

$$102.09 \times x + MW_{lca} \times y + 160.13 \times z + 1.0 \times (3 \times z - (x + y)) = b, \quad (3)$$

where x, y, and z represent the mole numbers (mmol) of the succinate, long-chain acyl group, and glucose unit of the succinylated polysaccharides, respectively; a is the volume (mL) of the 0.10 mol/L HCl aqueous solution required for neutralization. The DS_{lca} was obtained from the ^1H NMR results. MW_{lca} is the molecular weight of a long-chain acyl group, and b is the weight (mg) of the succinylated polysaccharide used for the test. The DS_{suc} was calculated from x/z. This measurement/calculation was repeated three times.

All chemicals and reagents are commercially available and were used without further purification. The paramylon, which was extracted from *Euglena gracilis* Strain EOD-1 (deposit number FERM BP-11530), was donated by KOBELCO Eco-Solutions Co., Ltd.

2.2. Synthesis of polysaccharide derivatives

The functional groups, degree of substitution values, and molecular weights of each product are shown in Tables S1 and S2.

2.2.1. Paramylon as starting material

A ^{13}C NMR spectrum of paramylon from *Euglena gracilis* Strain EOD-1 was consistent with those of β-1,3-glucans previously reported (Jin, Zhang, Yin, & Nishinari, 2006; Shibakami et al., 2013; Tamura, Wada, & Isogai, 2009). FT-IR was also measured: ^{13}C NMR (1.0 mol/L NaOD/D_2O, 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt as an internal standard) δ 106.3, 89.9, 79.6, 76.6, 72.3, 64.1; FT-IR (cm^{-1}) 3361, 2899, 1118, 1078, 1040, 885 (Fig. S2). ~100% purity of the paramylon was confirmed by the phenol–sulfuric acid method (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956). Weight average molecular weight (M_w) and number average molecular weight (M_n) of paramylon were determined by size-exclusion chromatography with multiangle laser light scattering (SEC-MALLS). The SEC-MALLS measurement was carried out on a DAWN HELEOS II multiangle laser photometer (Wyatt Technology) and an Optilab T-rEX refractive index detector (Wyatt Technology) equipped with a gel permeation chromatography column (Shodex KF-807L) (eluent 10 mmol/L LiBr in DMSO/DMF 75/25 (v/v), 1.0 mL/min, 50 °C). The solutions were purified using a 1.0-μm filter. The injection volume was 100 μL with a concentration of 1.0 mg/mL. The dn/dc value was 0.072. M_w 1.892×10^5, M_n 1.531×10^5.

2.2.2. Paramylon myristate (lower DS_{lca}) (**1a**)

To a homogeneous solution of paramylon (3.017 g, 18.61 mmol) in *N*,*N*-dimethylacetamide (DMAc) (150 mL) and lithium chloride (LiCl) (2.381 g, 56.18 mmol) prepared by heating at ~116 °C for 0.75 h were added dropwise (1.61 mL, 11.55 mmol) of trimethylamine (NEt_3) and 30 mL of a DMAc solution containing myristoyl chloride (0.234 g, 0.95 mmol) at room temperature. This mixture was heated at ~109 °C under a nitrogen atmosphere for 3 h. The reaction mixture was poured into a mixture of chloroform and methanol (100 mL/200 mL) to precipitate a white solid. This solid was separated by centrifugation. The resulting solid was then sequentially washed with a mixture of chloroform and methanol (100 mL/200 mL) and methanol (300 mL) for 15 min by mechanical stirring. Centrifugation produced a white gel-like solid. Air drying overnight and subsequent vacuum drying at 100 °C for 5 h produced paramylon myristate as a solid (3.303 g, 19.36 mmol, yield 104.0%). Successful preparation was confirmed by FT-IR measurement: FT-IR (cm^{-1}) 3313, 2882, 1607, 1363, 1119, 1043, 1031, 887.

2.2.3. Paramylon myristate (higher DS_{lca}) (**1b**)

A process similar to that described for the preparation of product (**1a**) was used to obtain a 93.6% yield of product (**1b**) (3.292 g, 17.32 mmol) from paramylon (3.000 g, 18.50 mmol) and myristoyl chloride (0.927 g, 3.76 mmol). Successful preparation was confirmed by FT-IR measurement: FT-IR (cm^{-1}) 3295, 2918, 1713, 1605, 1362, 1111, 1040, 1032, 887.

2.2.4. Paramylon palmitate (lower DS_{lca}) (**1c**)

A process similar to that described for the preparation of product (**1a**) was used to obtain a 103.6% yield of product (**1c**) (3.253 g, 19.32 mmol) from paramylon (3.022 g, 18.64 mmol) and palmitoyl chloride (0.257 g, 0.94 mmol). Successful preparation was confirmed by FT-IR measurement: FT-IR (cm^{-1}) 3326, 2881, 1606, 1362, 1111, 1042, 1031, 887.

2.2.5. Paramylon palmitate (higher DS_{lca}) (**1d**)

A process similar to that described for the preparation of product (**1a**) was used to obtain a 99.5% yield of product (**1d**) (3.356 g, 18.63 mmol) from paramylon (3.038 g, 18.73 mmol) and palmitoyl chloride (1.013 g, 3.68 mmol). Successful preparation was confirmed by FT-IR measurement: FT-IR (cm^{-1}) 3334, 2921, 1608, 1362, 1156, 1070, 1033, 889.

2.2.6. Paramylon stearate (lower DS_{lca}) (**1e**)

A process similar to that described for the preparation of product (**1a**) was used to obtain a 102.4% yield of product (**1e**) (3.204 g, 19.07 mmol) from paramylon (3.020 g, 18.63 mmol) and stearoyl chloride (0.286 g, 0.94 mmol). Successful preparation was confirmed by FT-IR measurement: FT-IR (cm^{-1}) 3334, 2919, 1607, 1361, 1159, 1044, 1032, 886.

2.2.7. Paramylon stearate (higher DS_{lca}) (**1f**)

A process similar to that described for the preparation of product (**1a**) was used to obtain a 104.1% yield of product (**1f**) (3.541 g, 19.35 mmol) from paramylon (3.012 g, 18.58 mmol) and stearoyl chloride (1.180 g, 3.89 mmol). Successful preparation was confirmed by FT-IR measurement: FT-IR (cm^{-1}) 3332, 2917, 2849, 1605, 1363, 1111, 1038, 883.

2.2.8. Curdlan stearate (lower DS_{lca}) (**1g**)

A process similar to that described for the preparation of product (**1a**) was used to obtain a 99.3% yield of product (**1g**) (3.217 g, 18.35 mmol) from curdlan (2.996 g, 18.48 mmol) and stearoyl chloride (0.283 g, 0.93 mmol). Successful preparation was confirmed by FT-IR measurement: FT-IR (cm^{-1}) 3304, 2917, 1605, 1363, 1112, 1042, 1032, 888.