Chemoenzymatic Synthesis of Biodegradable Polymers Containing Glucobiose Branches

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SUMMARY: Polymerizable sugar esters including glucobiose were synthesized from maltose or trehalose and divinyl adipate using enzyme in DMF. ^{13}C NMR data showed that 6'-position of maltose and mono-6-position of trehalose were esterified. Further, these glucobiose esters was polymerized with azo-initiator to give the corresponding polymers.

Introduction

Natural polysaccharides such as starch and cellulose are biomass existing abundantly on earth. Saccharide which is a good source of organic raw material was used in the development of high functional polymers such as environmental preservation materials, biofunctional materials, pharmaceuticals, etc. To utilize saccharide, several researchers have investigated polymers having sugar branch such as polystyrene containing *N*-acetyl-β-lactosamine moieties,[1)]

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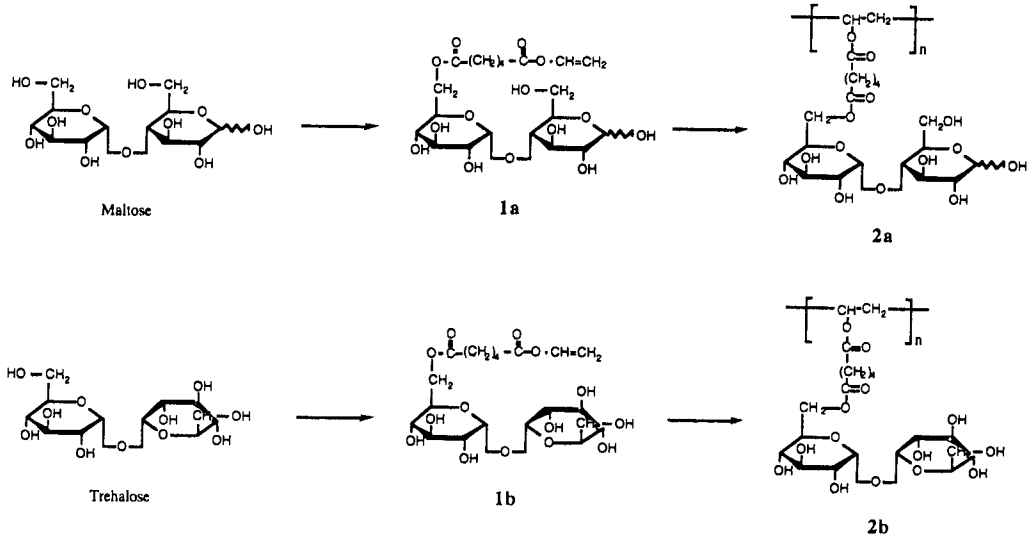
glucosyl oxyethylmethacrylate,[2] vinyl sucrose derivatives,[3] sucrose-based polyacrylate[4] and so on.[5-7] The main chains of these polymers are polystyrene and polyacrylate, which are known as non-biodegradable polymers. From the viewpoint of the preservation of the natural environment, it is important to develop new materials having biodegradability. Hence, we are examining sugar branched polymers having poly(vinyl alcohol) as a main chain, which are well known to have biodegradability.[8] Shibatani *et al.* reported the enzyme-catalyzed synthesis of polymerizable sugar ester, 6-*O*-vinyladipoyl-D-glucose,[9] and its polymerization to give polymer containing glucose branches.[10] The sugar branched polymers consist of three parts: the main chain, spacer arm and sugar



Scheme

moiety. Various types of the polymer can be obtained by changing these three parts. Disaccharides are renewable raw materials. In this study, we examined modification of sugar moiety, that is, synthesis

of the polymerizable glucobiose esters such as maltose and trehalose, and polymerization of resulting glucobiose esters with azo-initiator to give polymers having maltose or trehalose branch.

Experimental

Materials

Alkaline protease from *Streptomyces* sp. and lipoprotein lipase were from Toyobo Co. Lipase from *Alkaligenes* sp. was from Biocatalysts Ltd. Lipase type II from porcine pancreas was from Sigma. Divinyl adipate, azobis(2,2'-diamidinopropane)hydrochloride (ADPCL), maltose monohydrate and trehalose dihydrate were obtained from Wako Pure Chemicals. DMF and pyridine were obtained from Dojin Kagaku. The solvents were dried over 3Å molecular sieves (Wako Pure Chemicals) by shaking overnight.

Analytical methods

Glucobiose was detected by HPLC with refractive index measurements. A carbohydrate analysis column, TSK gel Amide-80 (TOSOH), was used with a mobile phase of a mixture of 75 % acetonitrile and 25 % water at a flow rate of 1.0 ml/min. TLC was performed on silica gel 60 F plates from Merck with a solvent consisting of ethyl acetate-methanol-water (17:4:2). The spots were developed by spraying with H_2SO_4, followed by heating. The positions of acylation in enzymatically prepared derivatives were established by ^{13}C NMR (JEOL:JNM-EX270). Infrared spectra were measured with an Infrared Spectro-photometer (Shimadzu:FTIR-8100M). Molecular weight of polymer was determined by gel permeation chromatography (GPC) with refractive index (TOSOH).

An analysis column, TSK G5000 PWXL +G4000 PWXL+G2500PWXL (TOSOH), was used with a mobile phase of 0.1M NaCl at a flow rate of 1.0 ml/min.

Enzymatic synthesis of polymerizable glucobiose esters

The reaction of maltose was initiated by adding 400 mg (2 mg/ml) of protease from *Streptomyces* sp. to 200 ml DMF solution containing 18 g (0.25 M) maltose and 79 g(2 M) divinyl adipate and stirred 7 days at 35 °C. The reaction was terminated by filtering off the enzyme and evaporating off the DMF. Purification was effected by silica gel chromatography with an eluent consisting of chloroform-methanol (8:1). 6'-*O*-Vinyladipoyl-maltose **1a** was obtained as a tan oil in a 17.3 g (70 %) yield. IRν_{max}(neat) cm^{-1}: 1730 (C=O), 1650 (vinyl). Anal. Calcd for $C_{20}H_{32}O_{14}$ (496.46): C,48.39; H: 6.50; O: 45.11. Found: C, 48.09; H: 6.53; O: 45.38. ^{13}C NMR data are shown in Tab.2.

The reaction of trehalose was initiated by adding 125 mg (5 mg/ml) of the protease to 25 ml DMF solution containing 2.36 g (0.25 M) trehalose and 5 g (1 M) divinyl adipate. The suspension was stirred 7 days at 35 °C. The reactions were terminated by filtering off the enzyme and evaporating off the DMF. Mono-6-*O*-vinyladipoyl-trehalose **1b** was obtained as a tan oil in a 1.27 g (41 %) yield. IRν_{max}(neat) cm^{-1}: 1740 (C=O), 1650 (vinyl). Anal. Calcd for $C_{20}H_{32}O_{14}$ (496.46): C,48.39; H: 6.50; O: 45.11. Found: C, 48.30; H: 6.33; O: 45.37. ^{13}C NMR data are shown in Tab.2.

Polymerization of glucobiose ester

The polymerization of 6'-*O*-vinyladipoyl-maltose **1a** was carried out

as follows: in a 10 ml sealed polymerization tube, a mixture containing 6'-*O*-vinyladipoyl-maltose (0.5g), ADPCL (5mg) and water (0.5g) was heated at 60 °C for 24 h. The resulting product was precipitated in acetone. The precipitated material was dried under reduced pressure at 40℃ to give poly(6'-*O*-vinyladipoyl-maltose) **2a** as a powder in 0.45 g (90%) yield. IRν_{max}(KBr) cm^{-1}: 1725 (C=O). ^{13}C NMR (DMSO-*d6*): δ 24.41, 33.95, 34.16 (-CH_2-), 61.35 (C-6α,β), 64.28 (C-6'), 70.53 (C-4'), 70.86 (C-5α), 71.14 (C-5'), 72.37 (C-2α), 72.96 (C-2'), 73.57 (C-3'), 74.83 (C-3α), 74.97 (C-2β), 75.73 (C-5β), 77.09 (C-3β), 81.28 (C-4β), 81.76 (C-4α), 92.69 (C-1α), 97.36 (C-1β), 101.71 (C-1'), 172.87, 173.35 (C=O). Mn=24,600, Mw/Mn=2.12.

The polymerization of mono-6-*O*-vinyladipoyl-trehalose **1b** was carried out by the same procedure to give poly(mono-6-*O*-vinyladipoyl-trehalose) **2b**. The precipitated material was obtained in a yield of 0.46 g of powder (92%). IRν_{max}(KBr) cm^{-1}: 1725 (C=O). ^{13}C NMR (DMSO-*d6*): δ 23.81, 24.08, 33.04, 33.40 (-CH_2-), 60.66 (C-6), 62.94 (C-6'), 69.44 (C-5'), 69.92 (C-4,4'), 71.34 (C-2), 71.57 (C-2'), 72.25 (C-5), 72.68 (C-3,3'), 93.31 (C-1'), 93.42 (C-1), 171.80, 172.59 (C=O). Mn=22,000, Mw/Mn=2.19.

Results and Discussion

Tab.1 shows the effects of the enzymes, which were reported to have transesterification activity of glucose in the previous paper, on maltose ester synthesis in DMF or pyridine. In pyridine, lipase from *Alcaligenes* sp. and protease from *Streptomyces* sp. were found to be

effective (conversion rate 69.6 % and 58.6 % respectively). Although *Alcaligenes* lipase showed lower transesterification activity in DMF than in pyridine, on the maltose ester synthesis, the protease showed higher activity (conversion > 99 %) in DMF. This result suggests the possibility that *Streptomyces* protease is useful for sugar ester synthesis in DMF, whether mono-saccharide or di-saccharide. Most sugars are hard to dissolve in non-polar organic solvents. DMF has better solubility against sugars. Hence the protease having high transesterification activity in DMF would be useful for enzymatic synthesis of sugar esters.

Tab.1. Effect of enzymes on the transesterification of maltose

Enzymes	Solvents	Conversion (%)
Lipase type II from porcine pancreas	Pyridine	29.6
Lipoprotein lipase from *Pseudomonas* sp.	Pyridine	1.3
Lipase from *Alcaligenes* sp.	Pyridine	69.6
Lipase from *Alcaligenes* sp.	DMF	41.0
Alkaline protease from *Streptomyces* sp.	Pyridine	58.6
Alkaline protease from *Streptomyces* sp.	DMF	99.9

The reactions were initiated by adding 100 mg/ml of lipases from *Alicaligenes* sp., porcine pancreas and *Pseudomonas* sp. or 5 mg/ml of alkaline protease from*Streptomyces* sp. to a solution containing 0.25 M maltose and 1 M divinyl adipate. The suspension was stirred for 24 hours at 35 °C. The reactions were terminated by filtering off the enzyme and the remaining glucobiose was detected by HPLC.

The enzymatic transesterifications of maltose and trehalose were carried out by using protease from *Streptomyces* sp. in DMF on a preparative scale. On the enzymatic synthesis of polymerizable glucobiose, 0.25 M maltose and 2 M divinyl adipate were dissolved

in DMF and the reaction was initiated by use of 2 mg/ml protease as described in the experimental section. On the other hand, 0.25 M trehalose and 2 M divinyl adipate could not be dissolved under the reaction conditions. Therefore 0.25 M trehalose and 1 M divinyl adipate was used in DMF instead. The time courses of these reactions are shown in Fig.1. In the synthesis of maltose ester a high conversion rate of more than 90 % was observed and reached a plateau in 2 days. In the case of trehalose, the conversion rate showed a gradual increase after 1 day and reached a plateau (60 %) in 7 days. Maltose and trehalose have two primary alcohols and even in the presence of high

Tab. 2. ^{13}C NMR data of mono-vinyladipate ester of maltose and trehalose

Position	Maltose[a]	Maltose-ester[a] **(1a)**	Position	Trehalose[b]	Trehalose-ester[b] **(1b)**
1α	92.98	92.93	1	93.19	93.43
1β	96.86	96.85			
2α	72.39	72.30	2	71.70	71.50
2β	75.10	74.98			
3α	74.33	74.28	3	72.99	72.89
3β	77.29	77.25			
4α	78.08	78.93	4	70.23	70.14
4β	77.87	78.74			
5α	71.06	71.08	5	72.55	72.62
5β	75.66	75.68			
6α	61.70	61.79	6	60.88	60.81
6β	61.87	61.90			
1'	100.76	100.98	1'		93.31
2'	72.76	72.70	2'		71.59
3'	73.97	73.80	3'		72.89
4'	70.45	70.63	4'		70.14
5'	73.77	71.51	5'		69.71
6'	61.60	64.65	6'		63.24

[a] Expressed in ppm relative to 3-(trimethylsilyl)propionic acid as an internal standard on D_2O.
[b] Expressed in ppm relative to tetramethylsilane as an internal standard on DMSO-*d6*.

concentration of divinyl adipate (4-8 times molar against sugar), the only main product of these reactions detected by TLC analysis (Rf

value 0.30) was mono-ester. It was assumed that these enzymes may not be able to recognize maltose mono-ester and further reaction may be difficult. Riva *et al.* also reported the enzymatic butylation of maltose by using protease from *Baccillus subtilis* in DMF, and that C-6 hydroxyl group of the tail glucose moiety (>95 % 6'-*O*-mono butyryl maltose) was esterified.[11] Mono-ester of maltose was obtained in high yield of 70 % (conversion rate 99%). On the other hand, trehalose mono-ester was obtained in yield of 41 % (conversion rate 60 %). Characterization of the products by ^{13}C NMR revealed that the esters were substituted at C6 position of terminal glucose

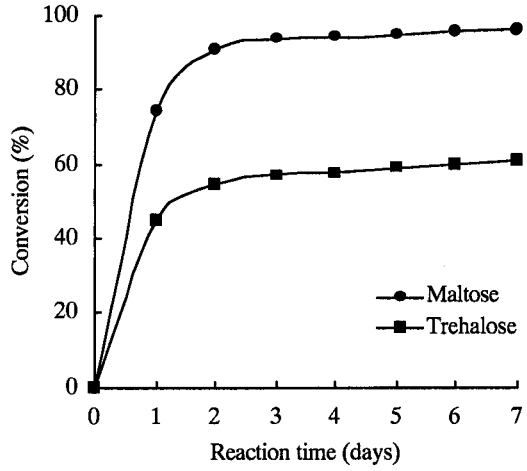


Fig. 1: Time course of polymerizable glucobiose ester synthesis catalyzed by alkaline protease from *Streptomyces* sp.

residue as shown in Tab.2. Thus signals of C-6' and C-5' positions for maltose ester shifted by 3.0 and -2.2 ppm, respectively compared

with maltose, but no shift of other positions for the ester were observed. While signals of C-6' and C-5' positions for trehalose ester alsoshifted by 2.4 and -2.9 ppm, respectively compared with trehalose, and also showed no shift of other positions for the ester. Yoshimoto *et al.* reported that after acylation of 6-position of glucose, the peak at C-6 shifted downfield and C-5 shifted upfield.[12] Furthermore, the TLC analysis of the reaction mixtures indicated that protease catalyzed mono-esterification only. These results showed that an ester bond was formed between the primary alcohol moiety (C-6') of maltose or trehalose and the carboxyl residue of vinyladipate, and that no esterification of the other primary alcohol (C-6) and the secondary alcohols were produced.

These polymerizable glucobiose esters were polymerized in water with azo-initiator and the corresponding polymer was obtained. Average molecular weights of the polymers having maltose and trehalose branch were 24,600 and 22,000, respectively, measured by GPC. IR spectroscopy revealed that vinyl group absorption disappeared, and ^{13}C NMR data were consistent with structure (see Experimental section). The hybrid materials by use of not only mono-saccharide but also di-saccharide are expected to find an interesting application. For example, sugar based surfactants by the use of disaccharide have been actively investigated, and these surfactants are biodegradable.[13] Furthermore, trehalose is a non-reducing glucobiose and it has recently become possible to produce trehalose effectively from starch on an industrial scale.[14] Trehalose is known to have a greater amount of unfrozen water than maltose.[15] In this experiment, we synthesized polymerizable glucobiose esters

catalyzed by protease, and subsequently polymerized it to give polymer containing glucobiose. These sugar branched polymers are expected to have interesting properties such as biodegradability and so on.

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