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Enzymatic Regioselective Synthesis of Vinyl Lactose Ester and Its Chemical Polymerization

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Abstract: Transesterification reaction of lactose with divinyladipate in pyridine catalyzed by an alkaline protease from *Bacillus subtilis* at 50°C for 3 days gave 6′-O-vinyladipoyl-lactose (yield 35%). Poly(6′-O-vinyladipoyl-lactose) with Mn = 21,200, Mw = 32,900, Mw/Mn = 1.56 could be obtained by chemical polymerization. Poly(vinyl alcohol) containing lactose branch was biodegradable. After 6 days in aqueous buffer (PH 7), this alkaline protease could degrade the polymer to an Mn of *ca.* 2100, Mw/Mn = 2.56.

Keywords: Regioselectivity, vinyl ester, lactose, alkaline protease, polymerization.

Regioselective acylation of carbohydrate is an attractive synthetic objective, but an arduous one because of the abundance of hydroxyl groups in their molecules, all of which are capable of being acylated by electrophiles[1]. A possible solution to multiple acylation is the use of specific blocking/deblocking steps. Unfortunately, the process is tedious and expensive. Unlike chemical catalysts, enzymes are high selective and enzyme-catalyzed regioselective acylation of sugars offers an alternative to the poor selectivity of chemical synthesis[2]. Noting the polymerizability of vinyl esters, various studies concerning transesterification of sugars with vinyl acrylate or divinyl dicarboxylates catalyzed by an enzyme were performed, and then the resultant vinyl esters were polymerized to yield sugar-based polymers[2-6]. Synthetic sugar-containing polymers have much potential for biocompatible and biodegradable materials[5].

In this paper, we investigated the transesterification of divinyladipate with lactose catalyzed by an alkaline protease from *Bacillus subtilis*, and the vinyl lactose ester was polymerized to obtain a novel, biodegradable polymer containing lactose branch.

Experimental

Materials

Alkaline protease from *Bacillus subtilis* was purchased from Wuxi Enzyme Co. Ltd. Divinyladipate was produced and purified as described by the patent literature[7]. The

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pyridine was dried over 3Å molecular sieves for 24 h *prior* to use. Lactose and all other chemicals were of the highest purity commercially available.

Analytical methods

The progress of reaction was monitored by TLC with an eluent consisting of ethyl acetate/methanol/water (75:25:5 by vol). Infrared spectra were measured with an infrared spectrophotometer (Nicolet: Nexus FTIR 670). The position of acylation in enzymatically prepared lactose ester was established by ^{13}C-NMR (Bruker AVANCE DMX 500). D_2O was used as a solvent. Molecular weight analysis was performed by gel permeation chromatography (GPC) with refractive index detection (Waters 2410) and Ultrahydrogel (Waters) GPC columns of 120 and 250 Å in series (Mw range from 100 to 80,000 daltons). The GPC columns were standardized with poly(ethylene glycol) with molecular weights from 400 to 89,500. The mobile phase was water (0.8 ml/min).

Transesterification reaction

20 mmol (6.84 g) lactose was dissolved in 200 mL pyridine containing 50 mmol (9.90 g) divinyladipate. The reaction was initiated upon addition of 1.0 g alkaline protease from *Bacillus subtilis* and the suspension was placed in a shaking incubator with a stirring speed of 250 rev./min for 3 days at 50°C. Filtering off the enzyme when the reaction was terminated. The pyridine was evaporated off. The product was isolated by silica gel chromatography with an eluent consisting of ethyl acetate/methanol/water (90:10:5 by vol) to give 6′-O- vinyladipoyl-lactose **1** (amorphous solid, yield 3.5 g, 35%).

Polymerization

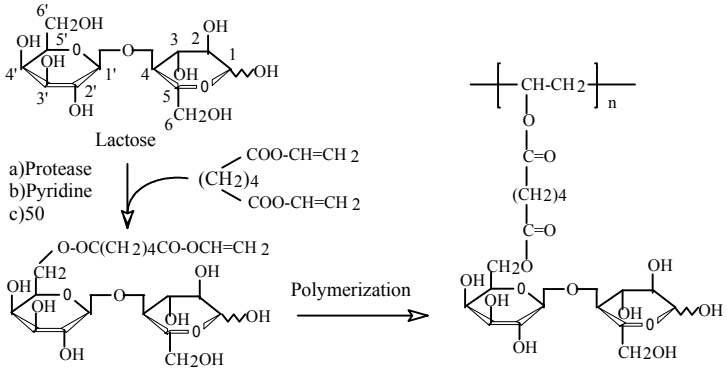
The polymerization of 6′-O-vinyladipoyl-lactose **1** was carried out by dissolving 0.4 g of vinyl lactose ester in 2 mL H_2O, and the solution was sparged with N_2 for 10 min. Potassium persulfate (2 mg) and H_2O_2 (3 mg) were added[2], and the solution was stirred at 60°C for 24 h. The resulting product was precipitated in acetone, filtered, and dried under vacuum at 45°C. The polymer **2** of 6′-O-vinyladipoyl-lactose was obtained in 77% recovered yield (0.30 g), Mn = 21,200, Mw = 32,900, Mw/Mn = 1.56.

Results and Discussion

Enzymatic synthesis of vinyl lactose ester and its chemical polymerization was shown in **Figure 1**. Initially the transesterification of lactose with divinyladipate catalyzed by alkaline protease from *Bacillus subtilis* in pyridine was compared with that catalyzed by lipase from *porcine pancreas* (PPL. Type II) (Sigma Chemical Co.). Interestingly, according to TLC analysis the transesterification reaction catalyzed with alkaline protease from *Bacillus subtilis* was more efficient than that catalyzed by PPL. So we had particular interest in the crude alkaline protease for its low cost and the possibility of industry application. On TLC analysis of reaction mixture, only one product was

detected. The vinyl lactose ester could be separated and purified by silica gel chromatography.

Figure 1 Enzymatic synthesis of vinyl lactose ester and its polymerization



The lactose ester was characterized by ^{13}C NMR analysis, as shown in **Table 1**. The general strategy was the same as described by Yoshimoto *et al*[8]. As established by them, acylation of a hydroxyl group of sugar results in a downfield shift of the peak corresponding to the O-acylated carbon and an upfield shift of the peak corresponding to the neighboring carbon. Characterization of the lactose ester **1** by ^{13}C NMR revealed that vinyl lactose ester was substituted at C-6′ position of lactose. Thus signals for C-6′ of lactose shifted downfield from 61.9 to 64.6 ppm and C-5′ position shifted upfield from 76.2 to 73.5 ppm compared with lactose.

Table 1 Chemical shifts of ^{13}C-NMR(D_2O) of lactose and lactose ester

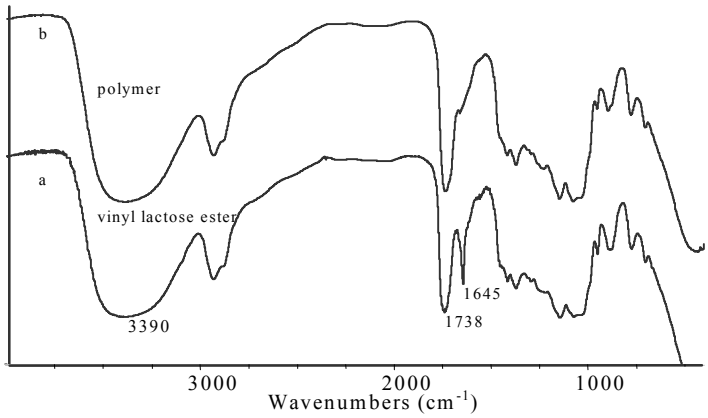
Carbon	Lactose	Lactose ester **1**	Carbon	Lactose	Lactose ester **1**
C-1′	103.8	103.9	C-2′	71.8	71.6
C-1β	96.6	96.6	C-2α	70.9	70.8
C-1α	92.7	92.7	C-4′	69.4	69.3
C-4α, 4β	79.4	80.3	C-6′	61.9	64.6
C-5′	76.2	73.5	C-6α, 6β	61.0	61.0
C-5β	75.6	75.5	$-CH_2-$		34.1, 33.9
C-3β	75.2	75.2			24.3, 24.2, 24.1
C-2β	74.7	74.7	C=O		176.9
C-3′	73.4	73.2			174.4
C-5α, 3α	72.2	72.2	$-CH=CH_2$		142.0, 100.2

This result implies that alkaline protease from *Bacillus subtilis* shows an effective regioselectivity in the transesterification of lactose with divinyladipate in anhydrous pyridine. FTIR spectrum of 6′-O-vinyladipoyl-lactose was shown in **Figure 2**. Assignment was as follows: 3390 cm^{-1} (ν_{O-H}), 1738 cm^{-1} ($\nu_{C=O}$), 1645 cm^{-1} ($\nu_{C=C}$).

The vinyl lactose ester was polymerized with potassium persulfate and H_2O_2 as

initiator to give poly(6′-O-vinyladipoyl-lactose) with Mn = 21,200. IR spectroscopy (**Figure 2**) of the polymer revealed that vinyl group absorption disappeared. The spectroscopic comparison result of vinyl lactose ester monomer and the resulting polymer confirmed vinyl group free radical polymerization. A number of potential applications of these novel polymers containing sugar branches including water absorbent materials, biocompatible polymers and hydrogels are being investigated in some groups[3-6].

Figure 2 FTIR spectra of 6′-O-vinyladipoyl-lactose and polymer



Y. Tokiwa *et al.* investigated the biodegradation of poly(6-O-vinyladipoyl-D-glucose) using the oxygen consumption method and found that the polymer was completely biodegradable[5]. In this work, biodegradability of the polymer containing lactose branch was examined as follows: 10 mg/mL polymer was dissolved in phosphate buffer, PH 7, and 2 mg/mL protease was added and the reaction was shaken at 37°C for 6 days. GPC analysis showed that the polymer was degraded from an Mn = 21,200, Mw/Mn = 1.56 to an Mn of *ca.* 2100, Mw/Mn = 2.56.

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