Biodegradation of a Sugar Branched Polymer Consisting of Sugar, Fatty Acid, and Poly(vinyl alcohol)

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ABSTRACT: We have examined synthesis of sugar branched polymer as a biodegradable material by utilizing natural substances. Biodegradation of a poly(vinyl alcohol) (PV−OH) having sugars and fatty acids was observed by a biochemical oxygen demand (BOD) tester using the oxygen consumption method. PV−OH is known as a biodegradable polymer by PV−OH assimilating microbes that are not distributed widely. In this paper, the sugar branched polymers were degraded depending upon the molecular weights, and the polymer having a molecular weight (M_n) of 3600 was degraded at 70−80% within 28 days by a soil or an activated sludge without addition of PV−OH assimilating microbes. This is because the proportion of PV−OH main chain in the molecular weight of the polymer is low. PV−OH with low molecular weight can be degraded without PV−OH assimilating microbes. These results indicate that the polymer is completely biodegradable and may have application as a new type of water-soluble material.

Introduction

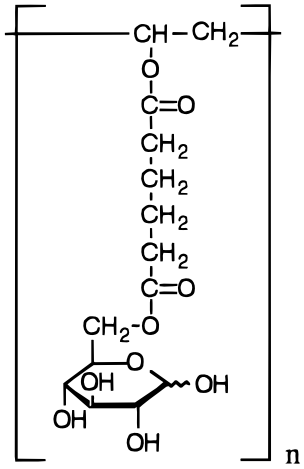
Synthetic polymers containing sugar branches have attracted considerable interest.[1] They have been the focus of intense research as new materials from the viewpoint of renewable supply in their application as surfactants,[2] in cell-recognition studies,[3] and as hydrogels.[4] Recently, the modification of methyl α-D-galactoside with vinyl acrylate was catalyzed by enzyme, and the resultant acryloyl sugar was chemically polymerized to yield a sugar-based polyacrylate.[5] Kobayashi et al. reported the chemical synthesis of polystyrene or polyacrylamide containing oligosaccharide moieties.[6] But the main chains of these polymers, such as polyacrylate, polyacryamide, and polystyrene, were not biodegradable. There are few reports about the sugar branched polymer having biodegradability. From the viewpoint of preservation of the natural environment, biodegradability is very important in the development of new materials.

Our studies involve the enzymatic synthesis of polymerizable sugar ester to avoid the use of protecting groups[7] and the subsequent chemical polymerization.[8] The sugar branched polymer consists of three parts, namely, poly(vinyl alcohol) (PV−OH) main chain which is known as the biodegradable polymer,[9] adipic acid ester moiety as a spacer arm, and glucose as a sugar moiety as shown in Scheme 1. We describe here the biodegradation of sugar branched polymers experimented by a biochemical demand (BOD) tester using the oxygen consumption method.

Results and Discussion

Figure 1 shows the biodegradation (BOD/TOD) of a polymer containing glucose branches and its constituents calculated from BOD values and theoretical oxygen demand (TOD) values. The BOD values were measured by using a soil obtained from a wooded area in Tsukuba City. Rapid degradations of glucose, adipic acid, and 6-*O*-vinyladipoyl-D-glucose were observed within the first 10 days, and poly(6-*O*-vinyladipoyl-D-glucose) with molecular weight of 7000 was degraded gradually to ca. 70% in 28 days. On the other hand, PV−OH with the degree of polymerization (dp) 1700 was degraded at low rate. PV−OH is well-known as a biodegradable polymer.[10] But only a restricted number of microbes can degrade PV−OH such as *Pseudomonas*,[9−11] *Bacillus megaterium*,[12] and *Alcaligenes faecalis*.[13] These PV−OH assimilating microbes are not widely distributed, and furthermore, the isolation of the microbes was carried out by the use of PV−OH with high molecular weight from 20 000 to 80 000. In this experiment, PV−OH with high molecular weight did not degrade, probably because the soil did not contain PV−OH assimilating microbes.There are a few reports about the biodegradation of PV−OH with low molecular weight. Only Matsumura et al. reported that PV−OHs having M_n of 2000 and 14 000 were biodegraded with river sediments and

Scheme 1



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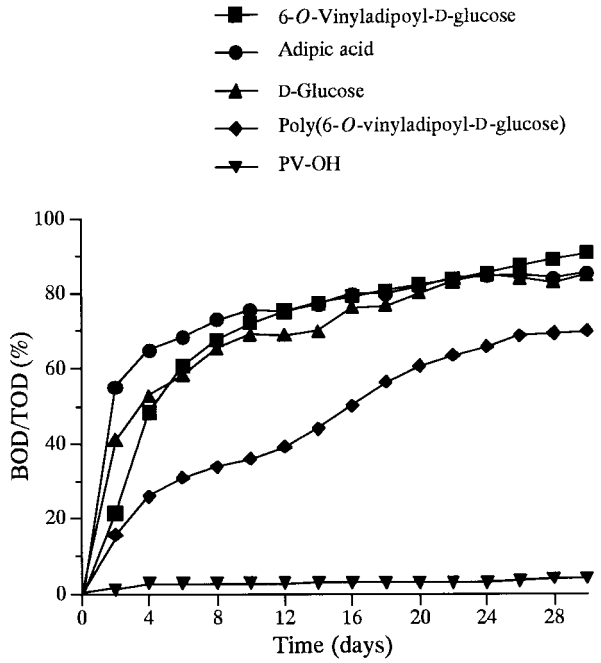

Figure 1. Biodegradability of poly(6-*O*-vinyladipoyl-D-glucose) and its constituents, calculated from biochemical oxygen demand (BOD) as measured by the oxygen consumption method in a BOD tester using a polymer concentration of 100 mg/L with soil at 25 °C, and the theoretical oxygen demand (TOD). poly(6-*O*-vinyladipoyl-D-glucose): M_n 7000, PV−OH: dp 1700.

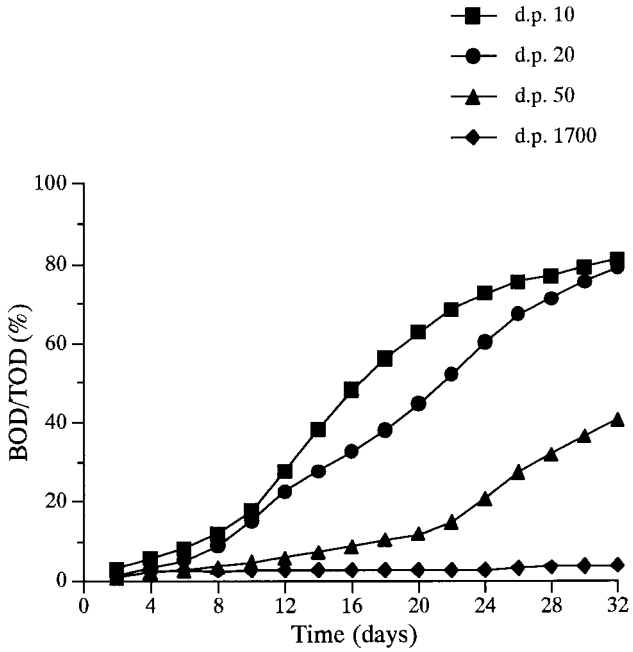

Figure 2. Biodegradability of poly(vinyl alcohol) having various molecular weights, calculated from biochemical oxygen demand (BOD) as measured by the oxygen consumption method in a BOD tester using a polymer concentration of 100 mg/L with soil at 25 °C, and the theoretical oxygen demand (TOD).

an activated sludge.[14] The sugar branched polymer consists of PV−OH, adipic acid, and glucose. The proportion of PV−OH main chain in the molecular weight of poly(6-*O*-vinyladipoyl-D-glucose) is ca. 13%, while the molecular weight of the PV−OH component of the polymer (M_n 15 000) is ca. 2000. Generally, materials with low molecular weight degrade easily compared with those with high molecular weight. Therefore, PV−OH with low molecular weight may be degraded without PV−OH assimilating microbes.

Figure 2 shows the degradation of PV−OH having dp of 10, 20, 50, and 1700. Biodegradation of these polymers increased with decreasing PV−OH molecular weight, and the degradation was 77%, 70%, 30%, and 5%, respectively, after 28 days. Matsumura et al. reported that PV−OH having M_n between 90 000 and 530 was well and equally biodegraded within 2 weeks by PV−OH assimilating microbes.[15] They observed no difference in the biodegradation of PV−OH by molecular weights and found that PV−OH assimilating microbes and enzymes required 3−5 vinyl alcohol monomer units to degrade.[15] In our experiment, PV−OH of high molecular weight did not degrade. This means that the soil did not contain PV−OH assimilating microbes, and we found that the degradation of PV−OH without addition of these microbes depends on the molecular weight.

PV−OH assimilating microbes produce a PV−OH degrading enzyme outside the microbe and uptake degraded fractions that are assimilated.[16] But PV−OH of low molecular weight must be readily taken into the microbe cells without degradation and then degraded by ubiquitous enzymes.

Biodegradation of poly(6-*O*-vinyladipoyl-D-glucose) having various molecular weights by a soil or an activated sludge is shown in Figure 3. The polymers with M_n of 3600 and 7000 were degraded to ca. 70% at 28 days by using a soil, and the polymers with M_n of 12 900 and 34 400 were of low degradation. In the case of using activated sludge (Figure 3B), biodegradation of these polymers depends more clearly on their molecular weight. Biodegradation decreased with increasing M_n, and the polymer with the highest M_n of 34 400 showed little degradation.

GPC is a convenient method for evaluating the degradation of a polymer. Figure 4 shows the GPC profiles of poly(6-*O*-vinyladipoyl-D-glucose) of various molecular weights. It was confirmed that the polymers of low molecular weight (M_n 3600, 7000, 12 900) were biodegraded under these conditions. Although the GPC peaks of the polymer with M_n of 34 400 showed no degradation, those of the polymers with M_n of 7000 and 12 900 decreased gradually, and the polymer fraction having M_n of 3600 completely disappeared after 4 weeks. These results are consistent with that of the BOD test, which showed that the polymer with M_n of 3600 has PV−OH main chain of M_n 500 and degraded completely after 28 days. The polymers with M_n of 7000 and 12 900 have PV−OH main chain of M_n 1000 and 1700, respectively, and while small peaks remained at 28 days, the peak heights decreased with passage of time. No degradation of the polymer with M_n of 34 400 was observed by BOD test or GPC pattern. In the previous report, we found that when the polymer (M_n 7000) eluted in the presence of 0.1 M NaCl, a single peak appeared on the GPC analysis; but in the absence of salt, the GPC pattern indicated several peaks in the higher molecular weight area, and the peak of the polymer was decreased.[17] It means that the polymer is aggregated in the absence of and/or low concentration of salt. This indicated that the polymer in an aggregated state may be related to the biodegradability.

In conclusion, it was found that degradation of poly(6-*O*-vinyladipoyl-D-glucose) depended on its molecular weight and was proved by experiment using a BOD tester and the GPC method that polymers having M_n

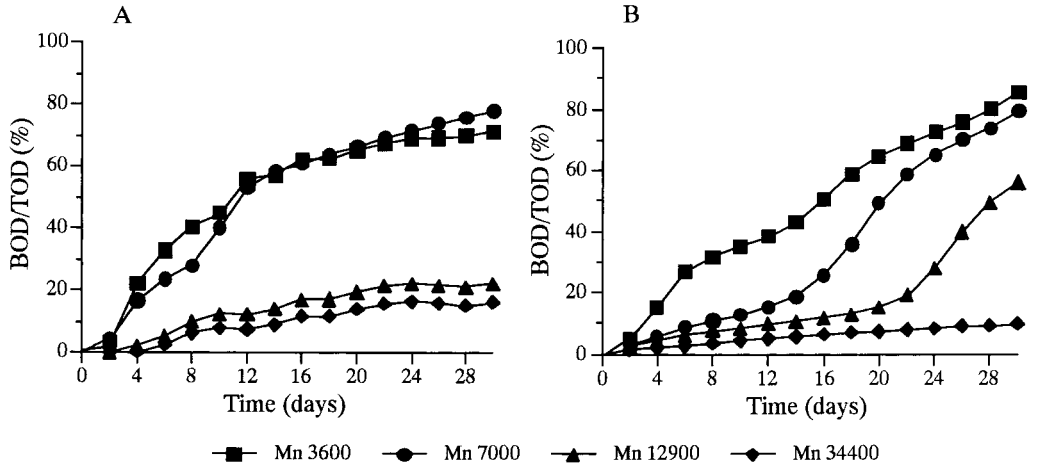

Figure 3. Biodegradability of poly(6-*O*-vinyladipoyl-D-glucose) having various molecular weights, calculated from biochemical oxygen demand (BOD) as measured by the oxygen consumption method in a BOD tester using a polymer concentration of 100 mg/L with soil (A) or activated sludge (B) at 25 °C, and the theoretical oxygen demand (TOD).

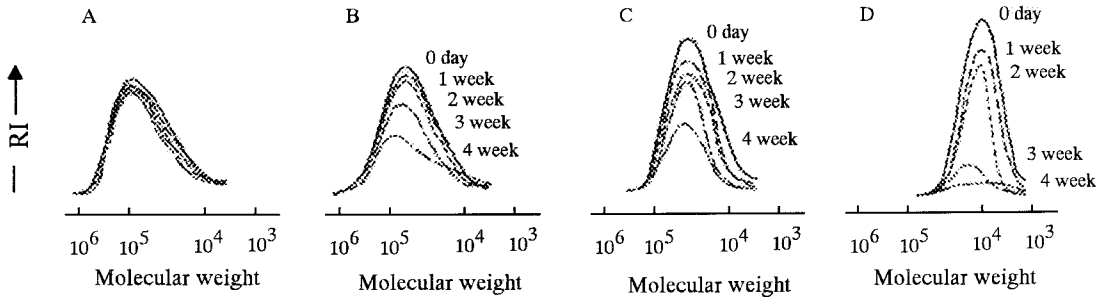

Figure 4. GPC profiles of poly(6-*O*-vinyladipoyl-D-glucose) having various molecular weights before and after biodegradation by soil suspension: (A) M_n 34 400, (B) M_n 12 900, (C) M_n 7000, (D) M_n 3600.

of less than 12 900 are degraded without addition of PV−OH assimilating microbes. These polymers would be easily biodegradable by microbes that are distributed widely.

Experimental Section

PV−OHs having dp of ca. 10, 20, 50, and 1700 (degree of saponification of 98−99 mol %) were donated by Kuraray Co., Ltd. (Osaka, Japan). 6-*O*-Vinyladipoyl-D-glucose was prepared by the enzymatic synthesis described in the previous reports.[8] Azobis(diamidinopropane) hydrochloride (ADPCL) was from Wako Pure Chemical Ind. Ltd (Osaka, Japan).

Synthesis of the Polymer Containing Sugar Branches. 6-*O*-Vinyladipoyl-D-glucose was polymerized using different solvents in DMF/water mixtures (0/100, 25/75, 50/50,75/25) to obtain polymers having various molecular weights. In a 10 mL sealed polymerization tube, a mixture containing 6-*O*-vinyladipoyl-D-glucose (0.5 g), ADPCL (2.5 mg), and water or DMF/water (2 mL) was agitated at 55 °C for 24 h. The resulting product was precipitated in acetone. The precipitated material was dried under reduced pressure at 40 °C to give about 90% of powder. IR (KBr): 1725 cm^{-1} (C=O). ^{13}C NMR (DMSO-d_6): δ 23.70, 23.90, 32.92, 33.30 ($-CH_2-$), 63.90 (C-6α, C-6β), 69.00 (C-5α), 70.14 (C-4α), 70.48 (C-4β), 72.04 (C-2α), 72.79 (C-3α), 73.39 (C-5β), 74.57 (C-2β), 76.28 (C-3β), 92.15 (C-1α), 96.76 (C-1β), 171.92, 172.63 (C=O). The molecular weights of the polymers were determined by gel permeation chromatography (GPC) with refractive index detection (HLC-8120GPC, TOSOH, Tokyo, Japan). An analysis column, TSK G5000 PWXL + G4000 PWXL + G2500PWXL (TOSOH, Tokyo, Japan), was used with a mobile phase of 0.1 M NaCl at a flow rate of 1.0 mL/min. Poly(ethylene oxide) (molecular weight fraction 26 000−920 000, TOSOH, Tokyo, Japan) and poly(ethylene glycol) (molecular weight fraction 600−20 000, Wako Pure Chemical Ind. Ltd, Osaka, Japan) were used as molecular weight standards. When the solvent of polymerization reactions was DMF/water at 0/100, 25/75, 50/50, and 75/25, the M_n were 34 400, 12 900, 7100, and 3600, respectively.

Biochemical Oxygen Demand (BOD) Test. BOD was determined with a BOD tester (OM8001A; Ohkura Denki Co., Tokyo, Japan) by the oxygen consumption method and basically according to the JIS standard guidelines (JIS K 6950) at 25 °C using a soil freshly obtained from a wooded area in Tsukuba City or an activated sludge from Kohoku municipal sewage treatment plant in Tsuchiura City. The incubation medium contained the following (mg/L): K_2HPO_4, 217.5; KH_2PO_4, 85.0; Na_2HPO_4, 260.5; NH_4Cl, 25.0; $CaCl_2 \cdot 2H_2O$, 36.4; $MgSO_4 \cdot 7H_2O$, 22.5; $FeCl_3 \cdot 6H_2O$, 0.25 (pH = 7.4). The concentration of polymers in the incubation medium was 100 mg/L.

Biodegradation Test in Shake Flask. The composition of culture medium was almost the same as in the BOD test described above. The polymer concentration was 1000 mg/L. The culture flasks were shaken with silicon caps at 25 °C, 130 rpm. The reaction mixtures before and after the biodegradation test were directly analyzed by GPC. An analysis column, TSK α-4000(TOSOH, Tokyo, Japan), was used with a mobile phase of dimethylformamide containing 10 mM lithium bromide at a flow rate of 0.3 mL/min and a column temperature of 40 °C. Polystyrenes (molecular weight fraction from 500 to 1 110 000, TOSOH, Tokyo, Japan) were used as molecular weight standards.

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