A Strategy for Increasing Molecular Weight of Polyester by Lipase-Catalyzed Polymerization

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A strategy for increasing the molecular weight of a polyester by lipase-catalyzed polymerization of a lactone and hydroxy acid was developed using azeotropic dehydration with toluene and a Dearn–Stark trap packed with molecular sieves. Thus, polycaprolactone (PCL) and poly(15-hydroxypentadecanoic acid) having number-average molecular weights of 81000 and 42000, respectively, were obtained by a 6-h polymerization at 70 °C.

Enzyme-catalyzed polymerization has been extensively studied for establishing green and sustainable chemistry in the field of polymer science.[1] It is revealed that lipase-catalyzed polymerization and degradation may become a novel tool for establishing a chemical recycling system for polyesters.[2–4] One of the tasks that should be developed is how to increase the molecular weight of polyesters produced by the lipase-catalyzed polymerization using industrially feasible monomers. In order to obtain polyesters with an ideal molecular weight, the factors that influence the enzymatic polymerization—reaction temperature, enzyme origin, reaction media, and water content—must be taken into account.[5]

The lipase-catalyzed polymerization of ε-caprolactone (ε-CL) was first reported by Uyama et al.[6] and Knani et al.[7] Polycaprolactone (PCL) having a molecular weight of a few thousands was obtained. Dong et al. recently reported that ε-CL was polymerized in 1,2-dichloroethane using molecular sieves at 45 °C to yield PCL having an M_n of 14500.[5] It has also been reported that 11-hydroxyundecanoic acid was polymerized in hexane in the presence of molecular sieves to yield poly(11-hydroxyundecanoic acid) having an M_n of 20000 for a 103-h reaction at 55 °C.[8] The positive effect of the addition of molecular sieves in the organic solvents was definitely observed; however, a longer polymerization time of 240 h was needed at 45 °C. A much shorter polymerization time will be needed for practical applications. The higher polymerization temperature, such as 70 °C, may increase the polymerization rate. Kumar et al. also reported the polymerization of ε-CL in toluene using lipase CA at 70 °C to yield PCL having an M_n of 44800 within a few hours.[9] However, a higher molecular weight PCL will be needed for practical use. At the higher temperature of 70 °C, the water absorption capability of the molecular sieves may decrease and also the transesterification reaction of PCL may occur by the action of molecular sieves. For these reasons, we used a toluene solution and Dean–Stark trap packed with molecular sieves where the liberated water produced by the polycondensation reaction was azeotropically removed and was absorbed on the molecular sieves. In this report, a strategy for increasing the molecular weight of the polyester was developed using azeotropic dehydration with toluene and molecular sieves in a Dean–Stark trap (Figure 1).

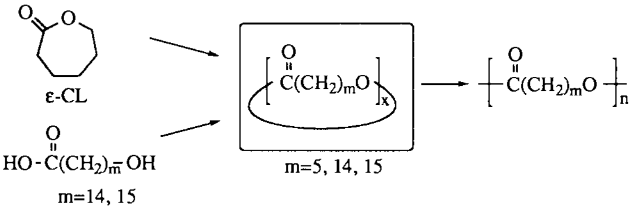

Figure 1. Polymerization of ε-CL, 15-hydroxypentadecanoic acid (m=14: 15-HPA) and 16-hydroxyhexadecanoic acid (m=15: 16-HA).

Table 1. Lipase-catalyzed polycondensation of hydroxy acids in toluene under azeotropic dehydration conditions[a]

Entry	Monomer	Yield/%	M_n	M_w/M_n
1[b]	15-HPA	0	---	---
2[c]	15-HPA	86	5,600	2.5
3	15-HPA	85	42,000	2.7
4	16-HHA	85	38,000	2.6

[a] Polymerization of hydroxy acid (300 mg) was carried out using 10% lipase CA (30 mg) at 30 °C for 6 h in toluene (1 mL) under azeotropic dehydration condition at 140–150 Torr and 70 °C. [b] Blank test without lipase CA. [c] Under atmospheric pressure.

It is reported that toluene is an effective solvent for the lipase-catalyzed polymerization of ε-caprolactone, and lipase CA showed the highest catalytic activities for the polymerization around 70 °C.[3,9] However, 15-hydroxypentadecanoic acid (15-HPA), for example, polymerized by lipase CA in toluene at 70 °C produced poly(15-HPA) having an M_n of 5600 as shown in Table 1.[10,11] This relatively low molecular weight may be ascribed to both the low water absorption capacity of the molecular sieves and transesterification of the resulting polymer caused by the molecular sieves at 70 °C.

In order to increase the molecular weight, 15-HPA was polymerized by lipase CA[12] in toluene at 70 °C under slightly reduced pressure during the azeotropic dehydration. That is, a mixture of 15-HPA (300 mg), 10% lipase CA (30 mg) and toluene (1 mL) was placed in a reaction flask (2 mL) equipped with stirring bar and a small Dean–Stark trap packed with activated molecular sieves 4A as the water absorbent and stirred at 140–150 Torr so as to provide a gentle reflux at 70 °C for 6 h. Thus, the liberated water was azeotropically transferred with toluene into the Dean–Stark trap. It was confirmed that the thus obtained poly(15-HPA) has an M_n of 42000 and the molecular weight was significantly increased compared to that without these dehydration procedures. The same results were obtained using 16-hydroxyhexadecanoic acid (16-HHA) in toluene at 70 °C as shown in Table 1. From the MALDI-TOF MS analysis, the polycondensation reactions of 15-HPA and 16-HHA produced macrocycles during the early stages of the polymerization. These macrocycles then further polymerized in a ring-opening manner to yield high-molecular weight poly(hydroxy acid)s.

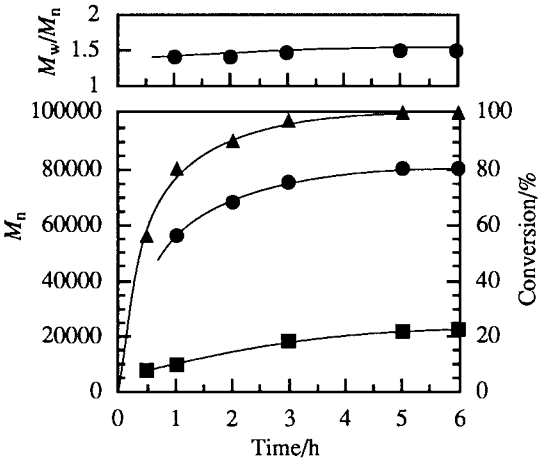

Figure 2. Time course of the polymerization of ε-CL in toluene using 10% lipase CA at 70 °C under azeotropic dehydration conditions at 140–150 Torr. ● : M_n, M_w/M_n, ▲ : Monomer conversion, ■ : M_n of PCL under atomspheric pressure

These macrocycles may be responsible to the formation of the high-molecular weight poly(hydroxy acid). In fact, it is reported that high-molecular weight poly(15-HPA) having an M_n of 86000 was produced by the polymerization of cyclic pentadecalactone using lipase CA in toluene.[10]

A significant molecular weight increase was observed for the polymerization of ε-CL by lipase CA in toluene. That is, ε-CL (300 mg) in toluene (1 mL) using 10% lipase CA (30 mg) was stirred and the reaction pressure was kept at 140–150 Torr so as to produce a gentle reflux to azeotropically remove the water that may contain in the lipase, by the molecular sieves in the Dean–Stark trap at 70 °C for 6 h. Figure 2 shows the time course of the polymerization of ε-CL in toluene using 10% lipase CA at 70 °C. It was found that the M_n of the resulting PCL under azeotropic dehydration conditions at 70 °C quickly increased, and after a 5-h polymerization, afforded a high-molecular weight PCL having an M_n of greater than 80000 with a monomer conversion of almost 100%. On the other hand, under atmospheric pressure without the azeotropic dehydration procedure, ε-CL slowly polymerized in toluene at 70 °C to yield a PCL having an M_n of about 20000 with a monomer conversion of almost 100%. These results showed the significant effect of the azeotropic dehydration during the lipase-catalyzed polymerization. In order to elucidate the polymerization profile, the polymerization products during the early stages were analyzed using MALDI-TOF MS and ^{1}H NMR with respect to the molecular structure[13]. Figure 3 shows the MALDI-TOF MS of the caprolactone (CL) oligomer obtained by the polymerization of ε-CL in toluene under azeotropic dehydration at 70 °C after a 0.5-h polymerization with a monomer conversion of 55%. The MALDI-TOF MS clearly showed the formation of repeating units having a mass of 114 *m/z*. Each peak of the MALDI-TOF MS corresponded to the macrocyclic structure with a Na^+ ion. On the other hand, without dehydration process, considerable amount of linear oligomer was produced by the reaction with water in lipase.

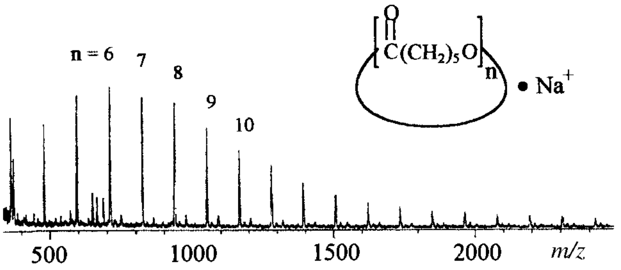

Figure 3. MALDI TOF MS spectrum of the CL oligomer obtained by the ring-opening polymerization of ε-CL in toluene by lipase CA under azeotropic dehydration at 70 °C after 0.5-h polymerization.

From these results, it is suggested that macrocycles are formed under azeotropic dehydration conditions by the lipase-catalyzed oligomerization of ε-CL or hydroxy acid in the early stages, and the macrocycles are then further polymerized by a ring-opening polymerization with lipase to produce high-molecular weight polyesters. These are summarized in Figure 1. It can be concluded that the first cyclization step of the azeotropic dehydration forming the cyclic oligomer was responsible for increasing the molecular weight in the lipase-catalyzed polymerization of both the lactones and hydroxy acids. Details of such mechanisms are now under study.

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11 The weight-average molecular weight (M_w), number-average molecular weight (M_n) and molecular weight dispersion (M_w/M_n) for the polymers were measured by size exclusion chromatography (SEC) using SEC columns (Shodex K-803L + K-8006 + K-800D, Showa Denko Co., Ltd., Tokyo, Japan) with a refractive index detector. Chloroform was used as the eluent at 1.0 mL/min.
12 Immobilized lipase from *Candida antarctica* (CA; Novozym 435) was kindly supplied by Novozymes Japan Ltd. (Chiba, Japan). The enzyme was dried in a vaccum over P_2O_5 at 25 °C for 1 day.
13 MALDI-TOF mass spectrum was measured on a Brucker Protein TOF in reflection mode using 2,5-dihydroxybenzoic acid as the matrix.