Single-Step Acylation of Polyester Terminals by Enzymatic Ring-Opening Polymerization of 12-Dodecanolide in the Presence of Acyclic Vinyl Esters

Hiroshi Uyama, Hirofumi Kikuchi, and Shiro Kobayashi*

Department of Materials Chemistry, Graduate School of Engineering, Tohoku University, Aoba, Sendai 980-77

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Enzymatic ring-opening polymerization of a 13-membered lactone, 12-dodecanolide (DDL), was performed in the presence of acyclic esters in bulk by using lipase catalyst. The acyl group was introduced at the terminal by adding a fatty acid vinyl ester. Effects of the chain length and concentration of the vinyl ester on the introduced ratio (functionality) and molecular weight of the polymer have been examined. The quantitative acylation of the terminal was achieved by using lipases derived from *Pseudomonas* family under appropriate reaction conditions. Methacryl- and ω-alkenyl-type polyester macromonomers were synthesized by the polymerization of DDL in the presence of vinyl methacrylate and vinyl 10-undecenoate, respectively. This process could be extended to single-step synthesis of polyester telechelics. The polymerization in the presence of divinyl sebacate produced a telechelic polymer having a carboxylic acid group at each end. In using a fatty acid ethyl ester or an acetic acid alkyl ester as additive, on the other hand, the quantitative introduction of the corresponding acyl group was not achieved.

Control of terminal structure of polymer is very important for design and preparation of functional polymers. Typical examples of terminal-functionalized polymers are macromonomers and telechelics. Various methodologies for synthesis of these polymers have been developed,[1—3] but elaborate and time-consuming procedures are often required.

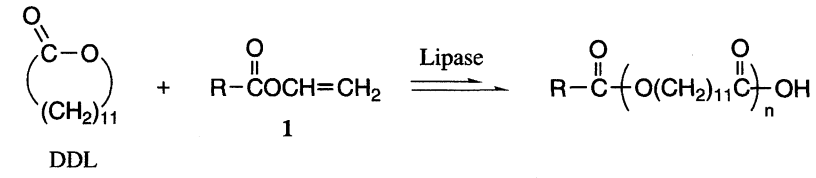
Recently, polyester synthesis through enzymatic catalysis has been extensively investigated.[4—18] Small-size (4-membered) and medium-size lactones (6- and 7-membered) as well as macrolides (12-, 13-, and 16-membered) have been enzymatically polymerized, yielding polyesters.[13—18] Macrolides show much lower reactivity and polymerizability than ε-caprolactone (ε-CL) due to their lower ring strain.[19,20] However, they are enzymatically polymerized much faster than ε-CL, probably due to the strong recognition of the macrolide by lipase.

Enol esters, typically vinyl esters, are often used as acylating agent for enzymatic esterifications and transesterifications,[21,22] since the leaving group, an unsaturated alcohol, irreversibly tautomerizes to a ketone or aldehyde. The reaction with the enol ester, therefore, produces the desired compound in higher yields, compared with those using an alkyl ester. We have used bis(enol ester)s as monomer for enzymatic polycondensation with glycols, yielding aliphatic polyesters under mild reaction conditions.[23]

Very recently, we have synthesized polyester macromonomers bearing a polymerizable methacryl or 10-undecenoyl group at the chain end by the enzymatic polymerization of 12-dodecanolide (13-membered, DDL) in the presence of a vinyl ester having the corresponding polymerizable group.[24] These macromonomers were obtained by a convenient, single-step procedure in a new type of macromonomer synthesis, in which the vinyl ester terminator was present from the beginning of the reaction. The present study deals with single-step preparation of acylated polyesters by the lipase-catalyzed polymerization of DDL in the presence of various vinyl and ethyl esters of fatty acids, and alkyl esters of acetic acid.

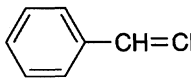
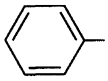
Results and Discussion

Enzymatic Polymerization of DDL in the Presence of Vinyl Esters. At first, the polymerization of DDL in the presence of vinyl laurylate (**1b**) (7.5 mol% based on DDL) was carried out using lipase derived from *Pseudomonas fluorescens* (lipase PF) in bulk at 60 °C for 120 h, neither stirring nor shaking, to produce the polymer in high yields (98%) (Scheme 1). The powdery enzyme was directly dispersed in the liquid mixture of the monomer and vinyl ester. If the enzyme was active, the white pellet polymer containing the dispersed enzyme was obtained. Figure 1 shows the ^{1}H NMR spectra of the polymer obtained by the polymerization using lipase PF in the absence and presence of vinyl laurylate. It was noted that the terminal structure of the polymer enzymatically obtained from DDL without the vinyl ester had a hydroxy group at one end and a carboxylic acid at the other,[16] which had a small characteristic peak (peak B) at $\delta = 3.6$ due to the α-methylene protons of the terminal hydroxy group (Fig. 1(A)). This peak completely disappeared in the spectrum of the polymer obtained in the presence of **1b** and a new triplet peak (peak F) was observed at $\delta = 0.9$ due to ω-terminal methyl protons of the fatty acid (Fig. 1(B)). Other



R= **1a**: $CH_3(CH_2)_{14}$- **1b**: $CH_3(CH_2)_{10}$- **1c**: $CH_3(CH_2)_6$- **1d**: $CH_3(CH_2)_2$-

1e: $CH_2=CH(CH_2)_8$- **1f**: $ClCH_2$- **1g**: $CH_2=C(CH_3)$- **1h**: $CH_2=CH$-

1i: $CH(CH_3)=CH$- **1j**:  **1k**: 

Scheme 1.

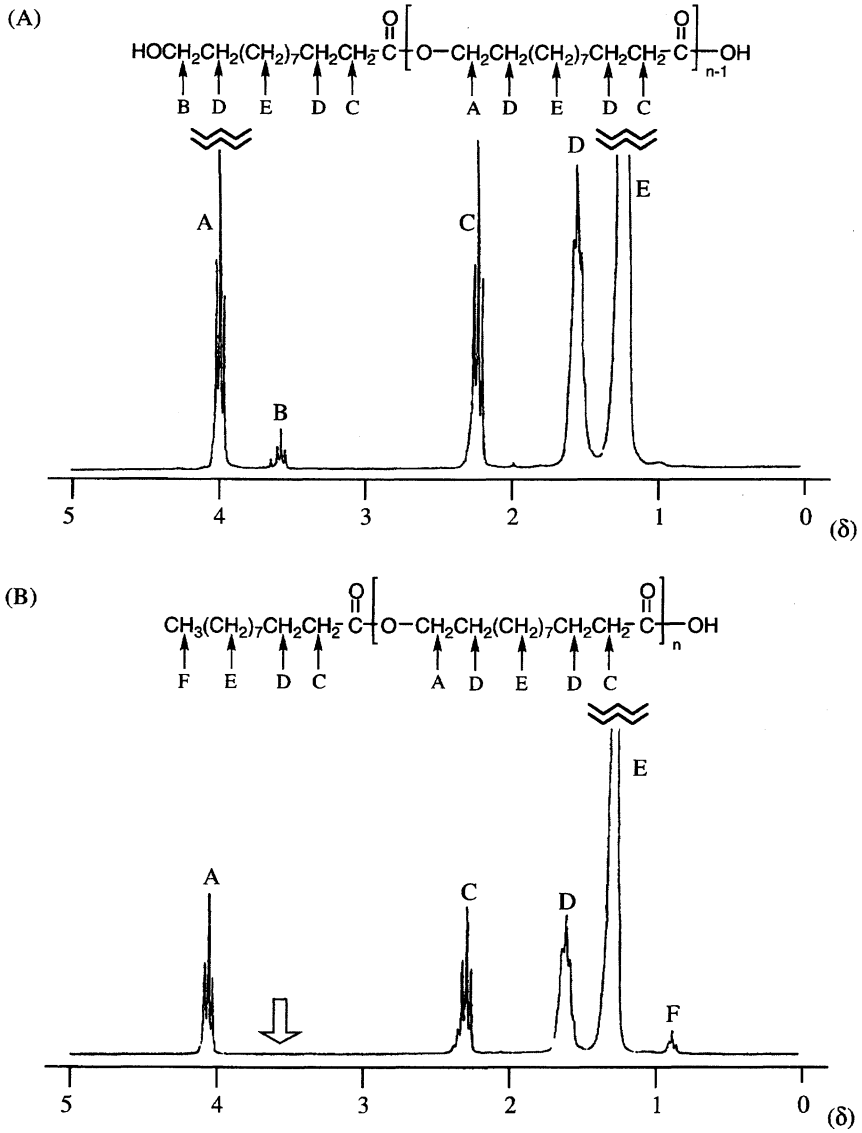


Fig. 1. ^{1}H NMR spectra of polyDDL obtained (A) in the absence of vinyl ester and (B) in the presence of vinyl laurylate (Entry 3 in Table 1).

peaks (peaks C—E), ascribable to the fatty acid moiety, are overlapping with those of polyDDL.

The terminal structure of the polyester was further confirmed by ^{13}C NMR spectroscopy. The spectrum of the polymer obtained without **1b** showed a small characteristic peak at $\delta = 63$ due to the α-methylene carbon of the terminal hydroxy group. In the spectrum of the polymer obtained in the presence of **1b**, this peak was not observed and a new

peak due to ω-terminal methyl carbon of laurylic acid ester appeared at $\delta = 14$. These data indicate that lauroyl group was quantitatively introduced at the polymer terminal.

Lipase origin normally affects the enzymatic reactions and polymerizations. In this study, three lipases showing high activity for the DDL polymerization were used as catalyst besides lipase PF: lipases derived from *Candida cylindracea* (lipase CC), from *Pseudomonas cepacia* (lipase PC), and from porcine pancreas (PPL). Polymerization results are summarized in Table 1. Lipase PC and PPL afforded the polymer in very high yield, but the polymer yield was low in using lipase CC. Functionality (the ratio of the polymerizable group introduced into the polymer terminal) was calculated from that of the integrated area between peaks B and F in Fig. 1. Lipases CC and PC were effective for the terminal functionalization: The functionality of the polymer obtained by using these enzymes was more than 95%. Quantitative introduction of the acyl group was only achieved using lipase PF. Therefore, this enzyme is used for all subsequent experiments throughout the present study.

Generally, lipase-catalyzed reaction behaviors are dependent upon alkyl chain lengths of substrates.[25] Here, effects of the chain length and concentration of the vinyl ester on the polymerization have been examined (Table 2). Vinyl palmitate (**1a**), **1b**, vinyl octanoate (**1c**), and vinyl butyrate (**1d**) were used as additives. Polymerization in the absence of the vinyl ester afford a polymer having molecular weight of 4100 quantitatively. This Mn value was larger than that obtained in the presence of the vinyl ester. The molecular weight decreased as the concentration of the vinyl ester increased. Using a low concentration of **1** ($\leq$5 mol% of DDL), the acyl group was not quantitatively introduced at the terminal alcohol, whereas quantitative acylation of the terminal alcohol was achieved when the concentration of **1a**, **1b**, or **1c** was more than 7.5%. The low yield of the polymer obtained by using 10% of the vinyl ester may be due to the decrease of the enzyme activity by acetaldehyde, which is formed by the transesterification of the vinyl ester.[26] The alkyl chain length of the vinyl esters hardly affected the yield and molecular weight of the polymer.

Besides fatty acid vinyl esters, vinyl 10-undecenoate (**1e**) and vinyl chloroacetate (**1f**) were used as additive. The 10-undecenoic acid moiety was quantitatively introduced in the case of a concentration of **1e** of more than 7.5% (Entries 15 and 16). The resulting polymer had a terminal unsaturated group, and hence can be used as ω-alkenyl-type macromonomer. On the other hand, the monomer was not polymerized in the presence of **1f**.

Table 2. Enzymatic Ring-Opening Polymerization of DDL in the Presence of Vinyl Esters (**1**) Using Lipase PF as Catalyst[a]

Entry	Vinyl ester	$[\mathbf{1}]_0/[\mathrm{DDL}]_0$	Yield[b]	M_n[b]	M_w/M_n[b]	Func.[c]
			%	$\times 10^{-3}$		
1	**1a**	0.050	98	3.7	1.9	0.75
2	**1a**	0.075	99	2.7	1.6	1.0
3	**1a**	0.10	53	1.5	1.8	>0.95
4	**1b**	0.025	99	4.0	2.2	0.62
5	**1b**	0.050	99	3.3	1.9	0.74
6	**1b**	0.075	98	2.8	1.6	1.0
7	**1b**	0.10	47	1.6	1.8	>0.95
8	**1c**	0.050	99	3.5	1.8	0.76
9	**1c**	0.075	92	2.6	1.6	>0.95
10	**1c**	0.10	34	1.4	1.4	>0.95
11	**1d**	0.050	96	4.2	2.1	0.71
12	**1d**	0.075	99	3.3	1.8	0.84
13	**1e**	0.025	100	3.7	2.0	0.33
14	**1e**	0.050	99	3.5	1.8	0.80
15	**1e**	0.075	98	2.4	1.5	>0.95
16	**1e**	0.10	37	1.9	1.5	>0.95
17	**1f**	0.075	0	—	—	—
18[d]	**1g**	0.075	98	3.7	1.8	0.57
19[d]	**1g**	0.10	92	3.6	1.7	0.83
20[d]	**1g**	0.125	79	3.3	1.7	0.95
21[d]	**1g**	0.15	62	2.9	1.7	0.95
22[d]	**1h**	0.075	0	—	—	—
23	**1i**	0.075	99	4.0	2.0	0.60
24	**1j**	0.075	77	2.9	2.2	0.95
25	**1k**	0.075	96	3.2	1.9	0.67

a) Polymerization of DDL (0.60 mmol) using lipase PF catalyst (0.030 g) in the presence of fatty acid vinyl ester in bulk at 60 °C for 120 h. b) Determined by GPC. c) Functionality, determined by ^{1}H NMR. d) Polymerization time: 72 h.

The enzymatic polymerization of DDL in the presence of α,β-conjugated acid vinyl esters, vinyl methacrylate (**1g**), vinyl acrylate (**1h**), vinyl crotonate (**1i**), vinyl cinnamate (**1j**), and vinyl benzoate (**1k**), was examined. By using **1g**, synthesis of a methacryl-type polyester macromonomer was attempted. By using more than 12.5% of the vinyl ester, the polymerizable methacyloyl group was almost quantitatively introduced at the polymer terminal. The concentration nec-

Table 1. Enzyme Screen for Ring-Opening Polymerization of DDL in the Presence of Vinyl Laurylate (**1b**)[a]

Entry	Lipase		Yield[b]	M_n[b]	M_w/M_n[b]	Func.[c]
	Origin	Code	%	$\times 10^{-3}$		
1	*Candida cylindracea*	lipase CC	23	1.0	1.4	>0.95
2	*Pseudomonas cepacia*	lipase PC	99	2.7	1.7	>0.95
3	*Pseudomonas fluorescens*	lipase PF	98	2.8	1.6	1.0
4	porcine pancreas	PPL	94	1.8	1.5	0.84

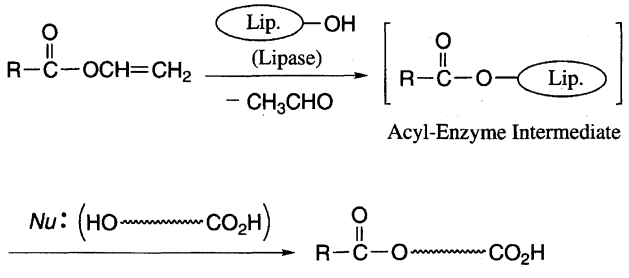
a) Polymerization of DDL (0.60 mmol) in the presence of **1b** (0.045 mmol) using lipase catalyst (0.030 g) in bulk at 60 °C for 120 h. b) Determined by GPC. c) Functionality, determined by ^{1}H NMR.

essary for the quantitative introduction of the ester was, thus, lager than for **1b**, probably due to the difference in the reactivity of **1b** and **1g** toward lipase. As the concentration of **1g** was increased, the molecular weight of the macromonomer decreased (Entries 18—21). The cinnamate group was almost quantitatively introduced at the terminal (Entry 24), whereas no polymerization occurred in using **1h** (Entry 22). Vinyl esters **1i** and **1k** resulted in the partial acylation of the polymer terminal (Entries 23 and 25). The difference of the polymerization behaviors, especially the yield and functionality of the polymer, may be due to that of the lipase-catalyzed reactivity of the vinyl esters used.

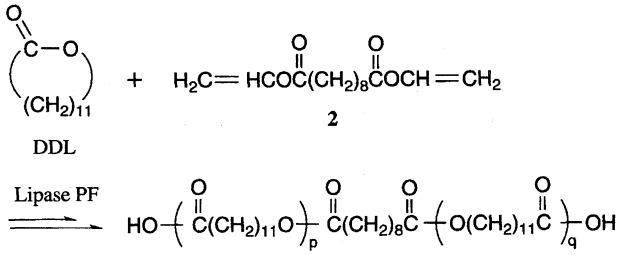
The mechanism of the acylation at the terminal may be explained as follows (Scheme 2). The catalytic site of lipase is a serine-residue and lipase-catalyzed reactions are well known to proceed via an acyl-enzyme intermediate.[27] In the lipase-catalyzed polymerization of lactones, the key step is the reaction of the lactone with lipase involving the ring-opening of the lactone, yielding the acyl-enzyme intermediate, which is nucleophilically attacked by the terminal hydroxy group of a propagating polymer in the propagating stage.[15] When a vinyl ester is present, the enzyme may form the acyl-enzyme intermediate from the vinyl ester. This intermediate is subjected to the reaction with the propagating end to give the polymer bearing the acyl group at the terminal. The resulting polymer has no hydroxy group, so the vinyl ester acts as a terminator.

Enzymatic Synthesis of Polyester Telechelics Having a Carboxylic Acid at Both Chain Ends. Hydroxy-terminated telechelic polyesters, prepared by ring-opening polymerization of ε-caprolactone in the presence of glycol, are industrially used as a soft segment of polyurethanes. On the other hand, there have been few reports on synthesis of telechelic polyesters bearing other functional groups at both ends. Recently, a carboxylic acid-terminated telechelic polyester was synthesized by ring-opening polymerization of ε-CL in the presence of succinic acid or a mixture of glycolic acid and succinic anhydride.[28,29]

Here, synthesis of a carboxylic acid-terminated telechelic polyester under mild reaction conditions was attempted by ring-opening polymerization of DDL in the presence of a bis(enol ester), divinyl sebacate (**2**) (Scheme 3). Polymerization results are shown in Table 3. The relationship between the concentration of the bis(enol ester) and the polymer molecular weight was similar to that using monomeric vinyl esters: the molecular weight decreased as a function of the concentration of **2**. When the concentration of **2** was 3.75%, the bis-acylation was almost quantitative at the polymer terminal to produce the telechelic polymer (Entry 4).



Scheme 2.



Scheme 3.

Table 3. Enzymatic Synthesis of Polyester Telechelics Bearing a Carboxylic Acid Group at Both Ends by Ring-Opening Polymerization of DDL in the Presence of Divinyl Sebacate (**2**)[a]

Entry	$[2]_0/[DDL]_0$	Yield[b] / %	M_n[b] / $\times 10^{-3}$	M_w/M_n[b]	Func.[c]
1	0.020	95	4.1	2.1	1.18
2	0.025	94	3.9	2.1	1.45
3	0.030	90	3.6	1.9	1.75
4	0.0375	41	2.9	1.9	1.95

a) Polymerization of DDL (0.60 mmol) using lipase PF catalyst (0.030 g) in the presence of divinyl sebacate (**2**) in bulk at 60 °C for 120 h. b) Determined by GPC. c) Functionality, determined by ^{1}H NMR.

Enzymatic Polymerization of DDL in the Presence of Other Acyclic Esters. The possibility of using other more readily available acyclic esters to modify the polyester terminal alcohol has been examined using acetic acid alkyl esters and fatty acid ethyl esters. Esters with different lengths of alkyl chain were utilized as additive.

The polymerization of DDL was carried out in the presence of acetic acid butyl, octyl, lauryl, or cetyl ester. The polymer was obtained almost quantitatively in the polymerization using 10% of the additive. The molecular weight was scarcely affected by the chain length of the ester. In all cases, the acyl group was not quantitatively introduced at the terminal (functionality$\approx$0.7), which is due to the lower reactivity of the alkyl ester toward lipase than that of **1**. As the concentration of the ester increased, the molecular weight decreased. The use of butyric, octanoic, laurylic, or palmitic acid ethyl ester as additive also resulted in the partial acylation of the polymer terminal.

Conclusion

Terminal acylation of aliphatic polyesters was attempted by enzymatic ring-opening polymerization of DDL in the presence of acyclic vinyl esters. The functionality was dependent on the structure and concentration of the vinyl ester as well as the lipase origin. The quantitative acyl group introduction was achieved from several vinyl esters under appropriate reaction conditions. Methacryl- and ω-alkenyl-type macromonomers were synthesized in the presence of

the corresponding vinyl esters. In all cases, the vinyl ester acted as terminator during the polymerization. This process could be applicable to syntheses of polyester telechelics with a carboxylic acid at both chain ends. The resulting terminal-acylated polymers were obtained by a single-step, convenient procedure under mild reaction conditions. In using other acyclic esters, acetic alkyl esters and fatty acid ethyl esters, however, the quantitative functionalization at the polymer terminal was not realized.

Experimental

Materials. 12-Dodecanolide (DDL) was purchased from Aldrich Chemical Co. DDL and acyclic esters were stored over freshly activated type 4 molecular sieves. Enzymes were employed without further purification. Lipase PC was donated by Amano Pharmaceutical Co. Lipases CC and PF were purchased from Biocatalysts. PPL was purchased from Sigma Chemical Co.

Enzymatic Ring-Opening Polymerization of DDL in the Presence of Acyclic Ester. A typical run was as follows (Entry 3 in Table 1). 0.12 g (0.60 mmol) of DDL, 0.010 g (0.045 mmol) of vinyl laurylate, and 0.030 g of lipase PF were placed in a dried tube and sealed. The tube was allowed to stand at 60 °C for 120 h. The reaction mixture was extracted with chloroform and part of the organic solution was separated by filtration. The filtrate was analyzed by gel permeation chromatography (GPC) for the determination of the yield and molecular weight of the polymer. The polymer was isolated by the reprecipitation procedure (chloroform as good solvent; methanol as poor solvent).

Measurements. GPC analysis was carried out using a Toso SC8010 apparatus with a refractive index (RI) detector under the following conditions: TSKgel G3000H_{HR} column and chloroform eluent at a flow rate of 1.0 mL min^{-1}. The calibration curves for GPC analysis were obtained using polystyrene standards. ^{1}H and ^{13}C NMR spectra were recorded on a 250 MHz Bruker AC-250T spectrometer.

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