Synthesis of low-molecular-weight copoly(L-lactic acid/ε-caprolactone) by direct copolycondensation in the absence of catalysts, and enzymatic degradation of the polymers

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Low-molecular weight copolymers of L-lactic acid (LA) and ε-caprolactone (CL) were synthesized by direct copolycondensation without catalysts at 200°C under a nitrogen atmosphere. The reaction proceeds by direct condensation between linear LA and linear 6-hydroxycaproic acid produced by hydrolysis of the cyclic CL. These copolymers were characterized with respect to molecular weight by gel permeation chromatography, composition by ^{1}H nuclear magnetic resonance (n.m.r.) spectroscopy, sequence by ^{13}C n.m.r. spectroscopy, and crystallinities by both differential scanning calorimetry and X-ray measurement. The morphology of copoly(LA/CL) can be subdivided into three states as a function of monomer composition according to the differences in T_g and crystallinity, e.g. solid (0–15 mol% CL composition ranges), paste (30–70 mol% CL composition ranges) and wax (85–100 mol% CL composition ranges). The *in vitro* degradation of the copolymer was examined by treatment in buffer solutions with and without enzymes and, as a result, *Rhizopus delemer* lipase showed the strongest degradation activity. In this case, the degree of enzymatic degradation is strongly dependent on the morphology of copoly(LA/CL), in which the pasty copolymer is much more subject to hydrolysis than the solid and waxy copolymers. For comparison, the *in vivo* degradation of the copolymer was investigated by implanting it subcutaneously in the back of rats.

(Keywords: low-molecular weight copolymers; L-lactic acid; ε-caprolactone; direct copolycondensation; amorphous polymer; crystalline polymer; *in vitro* degradation)

INTRODUCTION

The ring-opening homopolymerization of ε-caprolactone (CL) and ring-opening copolymerization of CL with other lactones, in the presence of various catalysts, have been studied by many workers to obtain high-molecular weight polyesters[1–8], which are well known to be typical biodegradable polyesters for application in biomedical fields[9–13], especially controlled drug delivery. In contrast, we have synthesized relatively low-molecular weight polyesters by direct polycondensation of α-hydroxy acids or lactones in the absence of catalyst and also studied their applications as biodegradable carriers for drug delivery systems, e.g. poly(D-lactic acid), poly(L-lactic acid) and poly(DL-lactic acid)[14,15], copoly(L-lactic acid/glycolic acid)[16], copoly(L-lactic acid/δ-valerolactone)[17,18], copoly(L-lactic acid/γ-butyrolactone)[19] and copoly(glycolic acid/lactones)[20]. The low-molecular weight polyesters are characterized by good biocompatibility because of no impurities, ease of shaping due to low softening point, ease of *in vivo* degradation control, and various morphologies such as solid, pasty and waxy states according to the monomer composition. In a series of studies, we have synthesized a low-molecular weight copolymer of L-lactic acid (LA) as a linear monomer and CL as a seven-membered ring monomer by direct polycondensation without catalysts. In this study, we report the reaction mechanism of copoly(LA/CL) deduced from results of studies by gel permeation chromatography (g.p.c.), differential scanning calorimetry (d.s.c.), ^{1}H nuclear magnetic resonance (n.m.r.) spectrometry, ^{13}C n.m.r. spectrometry and X-ray diffraction (x.r.d.) and also describe the enzymatic degradation mechanism, in order to apply it as a biodegradable carrier for drug delivery systems.

EXPERIMENTAL

Materials

L-Lactic acid (LA), 90% aqueous solution of monomer having 99% optical purity (C.V. Chemie Combinatie,

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Amsterdam), and ε-caprolactone (CL) (Tokyo Kasei Kogyo Co. Ltd) were used as polymerizable monomers.

Enzymes such as carboxylic esterase (EC 3.1.1.1) from porcine liver and lipases (EC 3.1.1.3) from wheat germ, hog pancreas and *Rhizopus delemer* were purchased from Sigma Chemical Co. The other chemicals were special grade.

Synthesis of copolymer

The synthesis of low-molecular weight copoly(LA/CL) was carried out by direct copolycondensation without using catalysts. Thus, a mixture of LA and CL with the desired composition was charged into a glass ampoule and then nitrogen gas was bubbled into the mixture at a flow rate of 200 ml min^{-1}. The ampoule was immersed in an oil bath maintained at 100, 150 or 200°C. In a homopolymerization system of CL, the reaction was done with 90 wt% aqueous solution of monomer, because no homopolymerization occurred in the absence of water.

Measurement of molecular weight

The molecular weight of copoly(LA/CL) was measured by both terminal carboxyl group analysis and gel permeation chromatography (g.p.c.).

In the terminal carboxyl group analysis, the number-average molecular weight ($\bar{M}_n$) of copoly(LA/CL), which was previously dissolved in benzyl alcohol, was determined by titration using 0.025 N benzyl alcohol solution of potassium hydroxide (KOH) and phenolphthalein as indicator. The resulting $\bar{M}_n$ was calculated from:

$$\bar{M}_n = \frac{1000W}{0.025f(V - V_0)} \quad (1)$$

where W is the weight of copolymer (g), f is the titre of 0.025 N KOH solution (titrated solution), V is the volume of titrated solution and V_0 is the blank volume of titrated solution, respectively.

Using equation (1), the number-average degree of polymerization ($\overline{DP}$) was estimated from:

$$\overline{DP} = \frac{\bar{M}_n - W_n}{M_{LA}(x) + M_{VL}(1-x)} \quad (2)$$

where W_n is the molecular weight of water, M_{LA} is the molecular weight of the lactyl unit (–$OCH(CH_3)CO$–), M_{VL} is the molecular weight of the ε-oxycaproyl unit (–$OCH_2CH_2CH_2CH_2CH_2CO$–) and x is the molar fraction of LA in the initial comonomer.

In g.p.c., the measurements were carried out with a Waters high-performance liquid chromatography, model ALC-244, at 25°C at a flow rate of 1 ml min^{-1} through 10^2, 10^3 and 10^4 Å Waters Ultrastyragel columns, in tetrahydrofuran. The number-average molecular weight ($\bar{M}_n$), the weight-average molecular weight ($\bar{M}_w$) and the molecular-weight distribution ($\bar{M}_w/\bar{M}_n$) of copolymers were calibrated by using standard polystyrene samples[21].

Instrumental analysis

The molar composition of copoly(LA/CL) was determined from 60 MHz ^1H n.m.r. spectra using a Hitachi R-600 FT spectrometer. The sequence analysis was obtained by 22.6 MHz ^{13}C n.m.r. spectra measured on a Hitachi R-90 FT n.m.r. spectrometer. For this purpose, the copolymer was dissolved in $CDCl_3$ with tetramethylsilane for internal reference, e.g. 5 wt% (^1H n.m.r.) and 20 wt% (^{13}C n.m.r.).

The crystallinity of the copolymer was obtained with a Rigaku X-ray diffractometer, using Ni-filtered Cu Kα radiation at 35 kV and 20 mA.

The melting point (m.p.) and glass transition temperature (T_g) of the copolymers were determined with a Seiko differential scanning calorimeter (d.s.c.), model DSC-10, at a heating rate of 5°C min^{-1}.

The optical rotation of the copolymer, $[\alpha]_D^{20}$, was measured by a Horiba polarimeter, model SEPA-200, using the D line of sodium at 20°C. For this purpose, the copolymer was dissolved in $CHCl_3$ (1 wt% concentration).

In vitro–in vivo degradation of copoly(LA/CL)

The *in vitro* degradation of solid, waxy and pasty copolymers was evaluated from the weight loss of the polymers after exposure to enzyme-containing buffer solutions. The *in vivo* degradation was studied by following the weight loss of samples subcutaneously implanted in the back of rats. Both solid-type (samples 1 and 2 in *Table 1*) and waxy-type (samples 6 and 7 in *Table 1*) copolymers were moulded into cylindrical specimens by the melt-pressing technique previously

Table 1 Direct copolycondensation of LA with CL in the absence of catalyst, and characteristics of copolymers

	Synthetic condition[a]			Property of copolymer													
	Monomer composition (mol%)				G.p.c.			D.s.c.		^1H n.m.r. Composition (mol%)			^{13}H n.m.r. Average block length				
No.	LA	CL	Reaction time (h)	$\bar{M}_n$[b]	$\bar{M}_n$	$\bar{M}_w$	$\bar{M}_w/\bar{M}_n$	T_g (°C)	M.p. (°C)	C_L	C_C	C_L/C_C	L_L	L_C	L_L/L_C	$[\alpha]_D^{20}$[c]	Appearance
1	100	0	12		3600	8800	2.44	44	142	100	0					−145	Solid
2	85	15	16		3700	8200	2.22	10	–	77	23	3.3	4.3	1.2	3.6	−98	Solid
3	70	30	16	2500	3600	7600	2.11	−16	–	61	39	1.6	2.6	1.6	1.6	−67	Pasty
4	50	50	18	±100	3300	7800	2.36	−39	–	44	56	0.79	2.0	2.4	0.83	−38	Pasty
5	30	70	16		3600	7900	2.19	−49	34	27	73	0.40	1.6	4.5	0.36	−18	Pasty
6	15	85	10		3700	7600	2.05	−54	44	16	84	0.19	1.5	9.7	0.15	−8	Waxy
7	0	100	4		5900	6800	1.15	ND[d]	59	0	100					0	Waxy

[a] Reaction was carried out at 200°C under a nitrogen atmosphere
[b] Terminal carboxyl group analysis
[c] $CHCl_3$ ($C = 1$)
[d] Not detected

reported[22]. In this, 50 mg of powdered copolymer was charged into poly(tetrafluoroethylene) tubing and then piston rods (stainless steel) were inserted from both sides of the tube under a pressure of 100 kg cm^{-2} at 40–50°C. Melting occurs, resulting in fine cylindrical specimens with a size of 2 mm diameter and 10 mm length. The pasty-type copolymers (samples 3, 4 and 5 in *Table 1*) were charged into a tubular container, which is characterized by ease of handling. The specimen-charging tube and specimen-charging container were sterilized by irradiating up to 30 kGy at −78°C (dry-ice temperature) with γ-rays from a ^{60}Co source. In this case, it was confirmed that the differences in such parameters as $\bar{M}_n$, $\bar{M}_w$, *in vitro* degradation and *in vivo* degradation between the sterilized and non-sterilized copolymers are of the order of a few per cent. A low irradiation temperature is essential to prevent severe degradation of the copolymer.

For *in vitro* experiments, 50 mg of the specimens pushed out from the tube or container was placed into flasks ($n=5$ per group) filled with 10 ml of a phosphate buffer solution, M/15 (pH 7.2), with or without 1 mg of enzyme. The media were exchanged every 24 h. The *in vitro* degradation experiments were carried out at 37 ± 1°C without stirring. After certain time intervals, the medium was removed from the flask and the samples were rinsed with fresh water, followed by weighing after vacuum drying to constant weight. The degree of *in vitro* degradation (per cent) was obtained from the ratio of the weight loss of the copolymer after treatment and the initial weight. In the *in vivo* experiments (two specimens per rat, two rats per group), 50 mg of the specimens was inserted subcutaneously in the back of male adult Wistar rats, weighing 350–400 g. After appropriate time intervals, the specimens inserted were excised from killed rats, preserved in 10% buffered formalin, pooled after being freed from surrounding connective tissues, dried *in vacuo* and then weighed.

RESULTS AND DISCUSSION

Copolymerization of LA and CL

The homopolymerization of CL was carried out in the presence of water without catalyst at temperatures of 100, 150 and 200°C under a nitrogen atmosphere. The changes in molecular-weight distribution of homopoly(CL) are shown in *Figure 1* as a function of reaction time. The polymerization rate of CL accelerates noticeably with an increase in reaction temperature. For example, the CL monomer peak at an elution volume of 30.7 ml did not disappear throughout an experimental period of 20 h when treated at a temperature of 100°C whereas it was completely missing after 7 h reaction at 200°C. This is because the cyclic CL monomer is converted into linear 6-hydroxycaproic acid (HCA) by hydrolysis, resulting in the appearance of peaks corresponding to oligomers followed by a single peak due to the formation of higher-molecular-weight polymer. In a copolycondensation system, the reaction of LA with HCA produced by hydrolysis of the cyclic CL was performed at 200°C. In this case, the CL monomer peak disappeared completely within 1 h by copolymerizing with LA. Such a quick disappearance may be related to the increase in acidity owing to LA monomer. The changes in molecular-weight distribution of copoly(LA/CL) are shown in *Figure 2* as functions of monomer composition and reaction time.

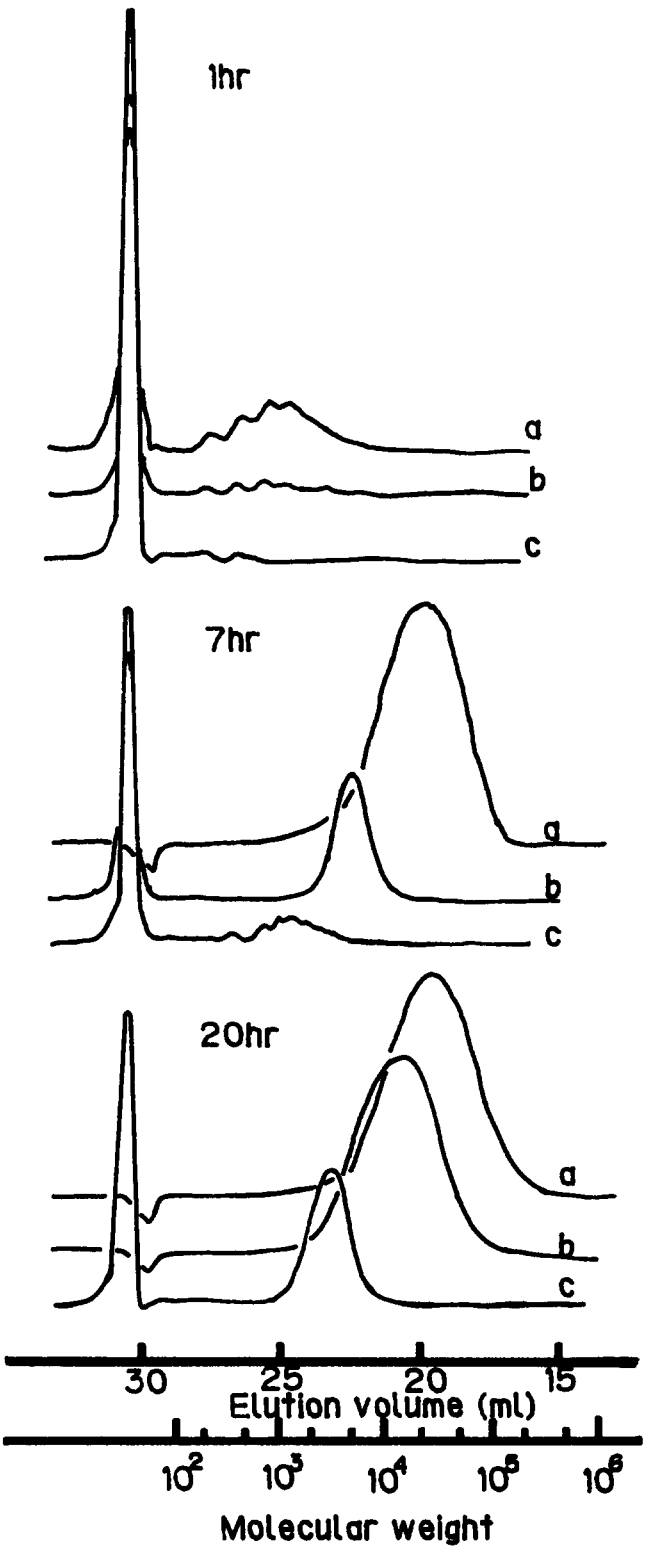

Figure 1 G.p.c. profiles of homopoly(CL), prepared by direct polycondensation in the presence of water without catalysts at temperatures of (a) 200°C, (b) 150°C and (c) 100°C, as a function of reaction time. Molecular weights given in figure refer to that with reference to standard polystyrene

The molecular-weight distributions of the copolymers led to peaks assigned to the oligomers during the first hour, but they gave a single peak with right-side shift because of the formation of higher-molecular-weight copolymer after 7 h reaction.

The effect of monomer composition on the degree of

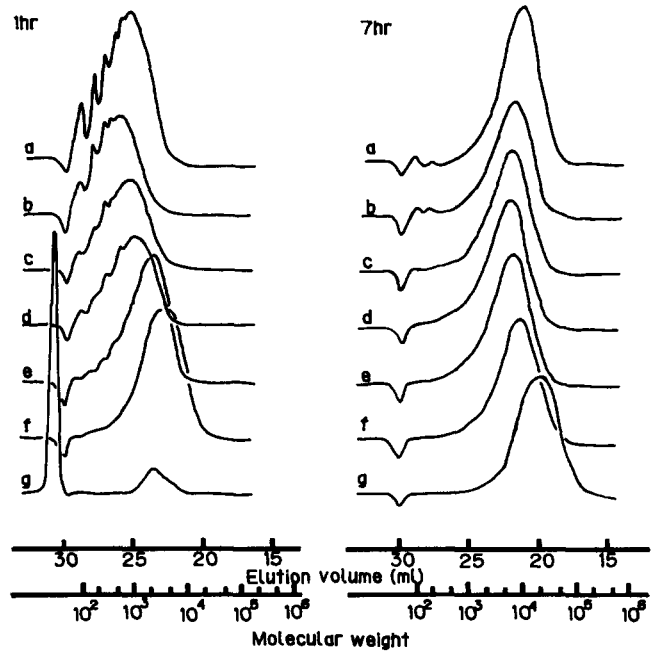

Figure 2 G.p.c. profiles of copoly(LA/CL) with monomer compositions of (a) 100/0, (b) 85/15, (c) 70/30, (d) 50/50, (e) 30/70, (f) 15/85 and (g) 0/100 mol%, prepared by direct copolycondensation without catalysts at 200°C, as a function of reaction time

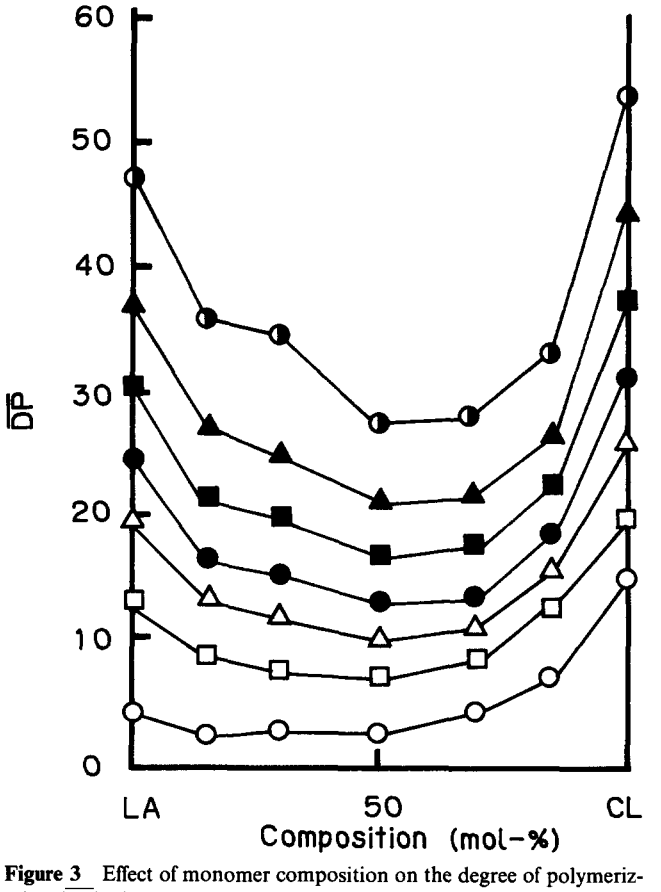

Figure 3 Effect of monomer composition on the degree of polymerization ($\overline{DP}$) of copoly(LA/CL), prepared by direct copolycondensation without catalysts at 200°C for periods of (○) 1 h, (□) 3 h, (△) 5 h, (●) 7 h, (■) 10 h, (▲) 14 h and (◑) 20 h

polymerization ($\overline{DP}$) of copoly(LA/CL) is shown in *Figure 3* as a function of reaction time. For this purpose, the unpolymerized CL monomer was removed from the system by dissolving in chloroform followed by reprecipitating with diethyl ether and drying *in vacuo*. $\overline{DP}$ is progressively increased with the passage of time for all monomer composition systems, but the $\overline{DP}$ increase for the LA/CL copolymer system is slower than that for each homopolymer system and, as a result, $\overline{DP}$ showed a minimum value at composition near 50 mol% CL. The cause of the appearance of such a minimum value may be related to the difference in polycondensability of pure monomers.

The methyl (CH_3), methylene (CH_2) and methine (CH) signals in 60 MHz ^{1}H n.m.r. spectra of homopoly(LA), copoly(LA/CL, 50/50 mol%) and homopoly(CL) are shown in *Figure 4*, measuring the molar composition of

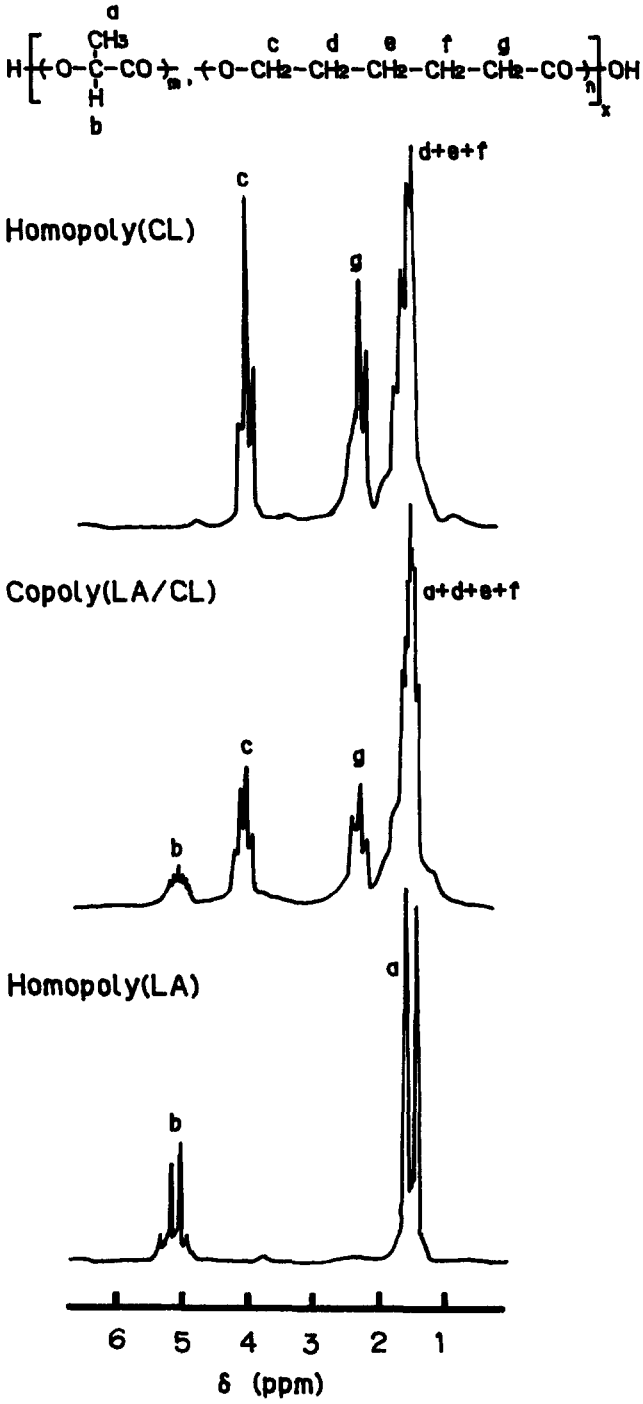

Figure 4 Methyl, methylene and methine signals in the 60 MHz ^{1}H n.m.r. spectra of a homopoly(CL) with $\bar{M}_n = 2600$ (sample 7 in *Table 1*), a copoly(LA/CL, 50/50 mol%) with $\bar{M}_n = 2500$ (sample 4 in *Table 1*) and a homopoly(LA) with $\bar{M}_n = 2500$ (sample 1 in *Table 1*)

the copolymer, in which the spectrum of the copolymer is markedly distinct from that of each homopolymer. Each resulting signal of the copolymer showed more multiple peaks than that of homopolymers. This finding means that the copolymer chain consists of LA and CL units. The molar composition of the copolymer was calculated from the ratio of areas for peaks (c+g) and peaks (a+d+e+f), as seen clearly in *Figure 4*. The CL contents in the monomers of 15, 30, 50, 70 and 85 mol% were found to be 23, 39, 56, 73 and 84 mol%, respectively. A small difference in molar composition between the initial monomer and the final product was observed, but it seems reasonable to conclude that CL monomer is quantitatively reacted with LA by direct copolycondensation in the absence of catalyst at 200°C. This reaction scheme is shown in *Figure 5*. The polycondensation of cyclic CL monomer proceeds through a linear HCA monomer produced by hydrolysis of CL. In this case, HCA is an unstable intermediate in the reaction system and, as a result, one can say that the rate of hydrolysis of the cyclic CL monomer is relatively slow in the presence of water, in contrast to a rapid rate of homopolycondensation for an unstable intermediate HCA. On the other hand, in a copolycondensation system, the rate of hydrolysis of CL is markedly accelerated by introduction of LA, which relates to the increase in acidity, giving quantitative reactivity between two linear monomers.

Sequence analysis of copoly(LA/CL)

The sequence analysis of copoly(LA/CL) was examined by ^{13}C n.m.r. spectroscopy according to the method proposed by Kricheldorf[23], in which the carbonyl (CO) signals are more suitable than other signals such as methyl (CH_3), methylene (CH_2) and methine (CH) signals, because of extreme sensitivity to sequence effects.

The CO signals in 22.6 MHz ^{13}C n.m.r. spectra of copoly(LA/CL) are shown in *Figure 6* as a function of monomer composition. Each homopolymer resulted in a single peak, in contrast to the multiple peaks for their copolymers. The CO signals of lactyl units assigned to the triad peaks are respectively split into four, four and three peaks in copoly(LA/CL) with monomer compositions of 70/30, 50/50 and 30/70 mol%, while the dyad peaks corresponding to the CO signals of ε-oxycaproyl units are all split into two peaks. On the basis of the above results, the dyad and triad sequences were assigned. The assignment can be seen clearly in *Figure 7*, which shows the dyad and triad sequences on the ^{13}C n.m.r. signals of lactyl and ε-oxycaproyl units in copoly(LA/CL). The peaks of the homogeneous bonds assigned to the δ values of each homopolymer are composed of peak B for the sequence C–C (C = ε-oxycaproyl unit) and peak A for the sequence L–L–L (L = lactyl unit), respectively (*Figure 6*). The peak B′ upfield from peak B corresponds to the dyads C–L and L–C. The peaks A′, A″ and A‴ are downfield from peak A, because the downfield shifts that the ε-oxycaproyl units exert on the central lactyl unit in the triads C–L–L and L–L–C are combined in the sequence C–L–C. In this case, the differentiation between peaks A′ and A″ is based on the consideration that the ε-oxycaproyl unit attached to the COOH group of the lactyl unit presumably causes greater downfield shift than the ε-oxycaproyl unit bonded to the OH group.

The average lengths of the homogeneous blocks were calculated by using equation (3) for the ε-oxycaproyl blocks (L_C) and equation (4) for the lactyl blocks (L_L)[23]:

$$L_C = \frac{I_{CC}}{I_{CL}} + 1 = \frac{I_{CC}}{I_{LC}} + 1 \quad (3)$$

$$L_L = \frac{I_{LLL} + I_{CLL}}{I_{LLC} + I_{CLC}} + 1 = \frac{I_{LLL} + I_{LLC}}{I_{CLL} + I_{CLC}} + 1 \quad (4)$$

where I_{CC} is the intensity of a peak representing the C–C

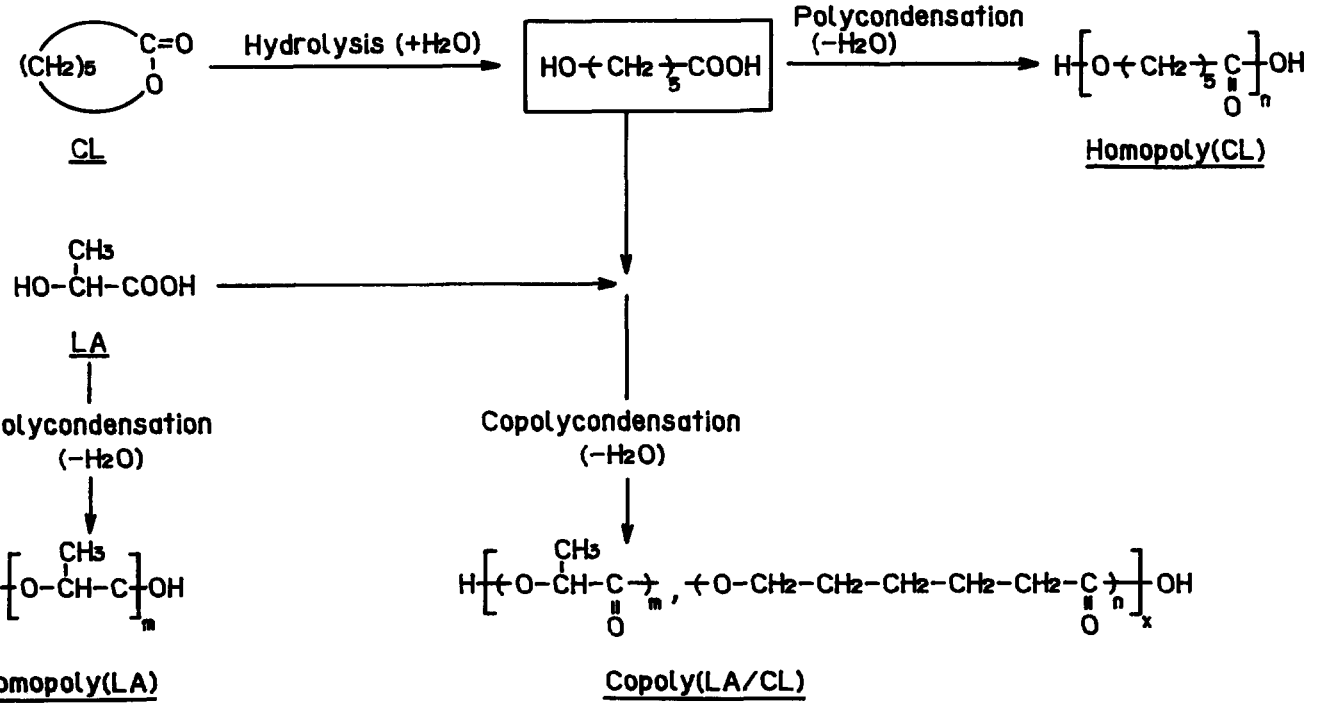

Figure 5 Reaction scheme for the direct copolycondensation of LA and CL in the presence of water without catalysts

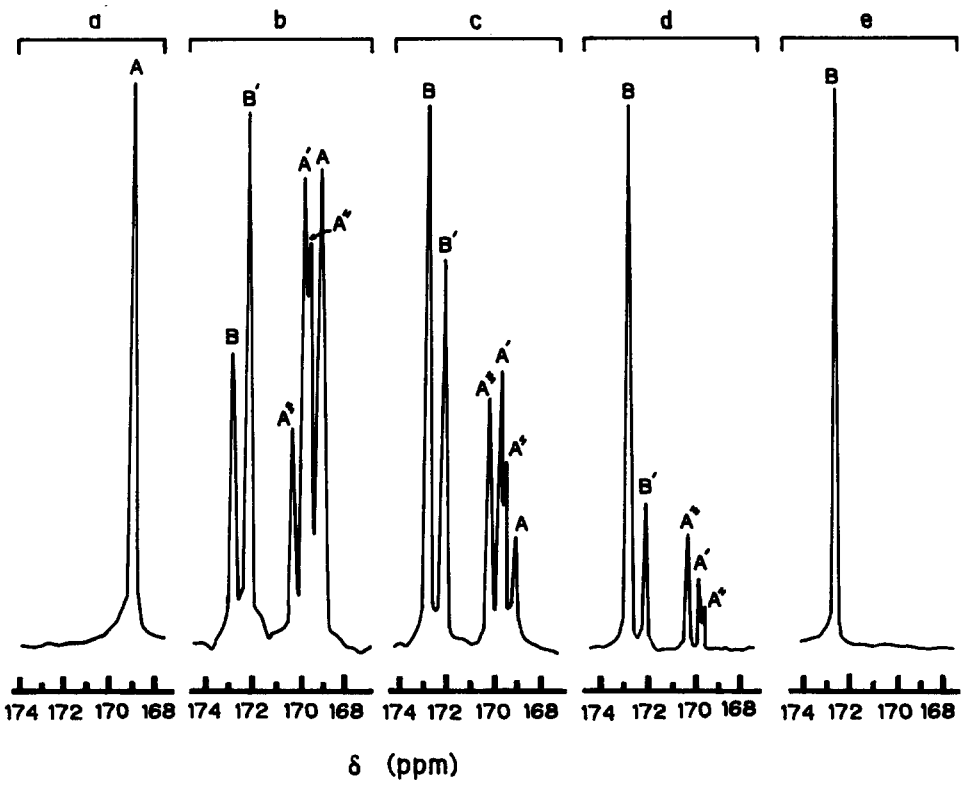

Figure 6 Carbonyl signals in the 22.6 MHz ^{13}C n.m.r. spectra of copoly(LA/CL) with monomer compositions of (a) 100/0, (b) 70/30, (c) 50/50, (d) 30/70 and (e) 0/100 mol%. The experimental conditions were the same as those in *Table 1*

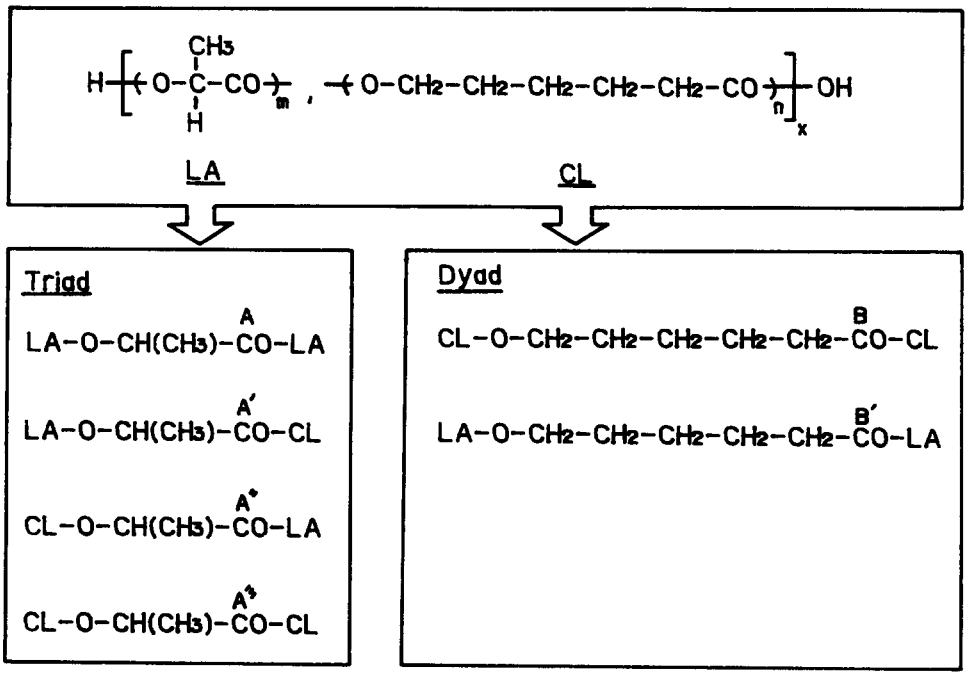

Figure 7 Dyad and triad sequences on the ^{13}C n.m.r. signals of lactyl and ε-oxycaproyl units in copoly(LA/CL)

dyad (peak B in *Figure 6*), I_{CL} (or I_{LC}) is the intensity of a peak representing the C–L dyad (or L–C dyad) (peak B′ in *Figure 6*), I_{LLL} is the intensity of a peak representing the L–L–L triad (peak A in *Figure 6*), I_{LLC} and I_{CLL} are the intensities of the peaks representing triads of two lactyl units and one ε-oxycaproyl unit (peaks A′ and A″ in *Figure 6*) and I_{CLC} is the intensity of a peak representing the C–L–C triad (peak A‴ in *Figure 6*), respectively. The L_C and L_L values estimated according to equations (3) and (4) are listed in *Table 1*. These L_L/L_C ratios must be identical with the molar compositions of the copoly-(LA/CL), which were determined from ^1H n.m.r. spectra, so that the quantification of ^1H n.m.r. signals makes it possible to check the correctness of ^{13}C n.m.r. signal

intensities as shown by:

$$(C_L/C_C)(^1\text{H n.m.r.}) = (L_L/L_C)(^{13}\text{C n.m.r.}) \quad (5)$$

where C_L and C_C are the LA and CL molar compositions in the copolymers determined by ^{1}H n.m.r. spectroscopy, respectively. The C_L/C_C ratios (^{1}H n.m.r.) are in fair agreement with the L_L/L_C ratios (^{13}C n.m.r.), as shown in *Table 1*, supporting the validity of each signal intensity. A sequential block copoly(LA/CL) with relatively equal blocks (L_L and L_C are 2.0–2.4) is expected at a monomer composition of 50/50 mol% LA/CL. On the contrary, sequential block copolymers with relatively long lactyl or ε-oxycaproyl blocks can be expected respectively at higher LA or CL composition, but the homogeneous blocks of lacyl units in the copolymers are generally difficult to elongate compared with the ε-oxycaproyl units, e.g. the LA/CL ratios were found to be 4.3/1.2 for copoly(LA/CL, 85/15 mol%) and 1.5/9.7 for copoly-(LA/CL, 15/85 mol%), respectively. This is due to higher reactivity of CL in the copolycondensation system.

Crystallinity of copoly(LA/CL)

The d.s.c. measurements were performed to check the crystallinity of copoly(LA/CL) and the results are shown in *Figure 8* as a function of monomer composition. The d.s.c. pattern of a LA homopolymer shows a T_g of 44°C, followed by a crystalline exotherm at 95°C and a melting endotherm at 142°C (*Figure 8a*), in contrast to only a melting endotherm at 59°C for a CL homopolymer because of high crystallinity (*Figure 8f*). Crescenzi *et al.*[24] determined the enthalpy of fusion (ΔH) of 100% crystalline homopoly(CL) by means of d.s.c., which gives a ΔH value of 139.5 J g^{-1}. Using this value, the crystallinity of homopoly(CL), which was obtained by direct poly-condensation in the presence of water without catalysts for 4 h at 200°C (sample 7 in *Table 1*), was estimated, and it was found to be 76%. In a copoly(LA/CL) system, the copolymers ranging in composition from 85 to 70 mol% CL show both T_g and melting endotherms, in contrast to only T_g for the 50–15 mol% CL-containing copolymers, which are amorphous. The cause of formation of amorphous copolymers is not clear at present, but possibly it may be related to the formation of sequential copolymers with mutually short blocks of lactyl and ε-oxycaproyl units. The T_g of copoly(LA/CL) is listed in *Table 1* and, as can be seen, T_g rapidly decreases with increase in CL content in the copolymer. This is due to the increase in mobility of ε-oxycaproyl units in the copolymer chain, in which the five methylene groups act

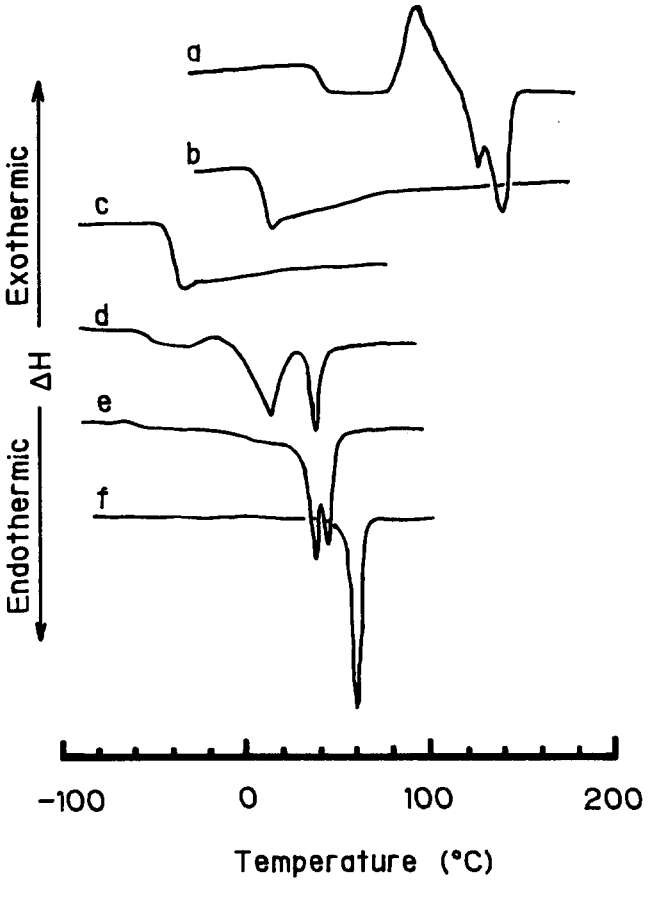

Figure 8 D.s.c. curves of copoly(LA/CL) with monomer compositions of (a) 100/0, (b) 85/15, (c) 50/50, (d) 30/70, (e) 15/85 and (f) 0/100 mol%. The experimental conditions were the same as those in *Table 1*

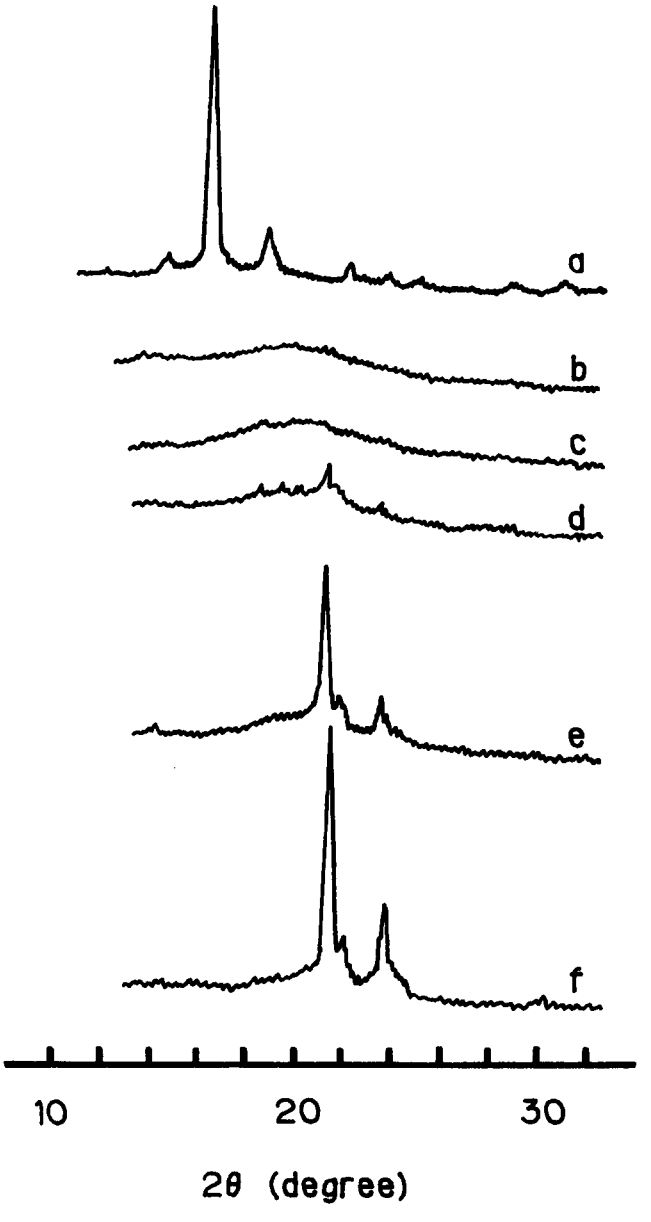

Figure 9 X-ray diffraction patterns of copoly(LA/CL) with monomer compositions (a)–(f) as given in *Figure 8*. The experimental conditions were the same as those in *Table 1*

as soft segments. The morphologies of copoly(LA/CL) obtained in this study can be subdivided into three types according to the differences in T_g and crystallinity: solid state for CL composition from 0 to 15 mol%, pasty state for CL composition from 30 to 70 mol%, and waxy state for CL composition from 85 to 100 mol%.

The X-ray diffraction patterns of the LA/CL copolymers with $\bar{M}_n = 2500$ are shown in *Figure 9* as a function of monomer composition. The homopoly(LA) was annealed for 15 h at 95°C *in vacuo* (10^{-3} mmHg) before X-ray measurement because it showed the crystallization exotherm as seen in *Figure 8a*. Similar treatment was performed in a 85/15 mol% LA/CL copolymer system. The copolymers with LA/CL compositions of 100/0, 30/70, 15/85 and 0/100 mol% show reflections of crystal lattices, e.g. $2\theta = 14.7°$, 16.6° and 19.0° for a homopolymer of LA (*Figure 9a*), $2\theta = 21.2°$ and 23.5° for a 30/70 mol% LA/CL copolymer (*Figure 9d*), $2\theta = 21.3°$, 21.9° and 23.6° for a 15/85 mol% LA/CL copolymer (*Figure 9e*) and $2\theta = 21.4°$, 22.0° and 23.7° for a homopolymer of CL (*Figure 9f*), respectively. However, such crystalline reflections disappeared for the 85/15 and 50/50 mol% LA/CL copolymers (*Figures 9b* and *9c*), owing to the formation of amorphous copolymers. This agreed closely with results from the d.s.c. measurements.

In vitro–in vivo degradation of copoly(LA/CL)

It is well known that the homopolymers of LA and CL are hydrolysed non-enzymatically and enzymatically, because they have ester bonds (–COO–) in the main chain[15,25–27]. The effect of monomer composition on the *in vitro* degradation of copoly(LA/CL) with $\bar{M}_n = 2500$, which was treated at 37°C for 3 weeks in various enzyme-containing buffer solutions, is shown in *Figure 10*. This copolymer was degraded non-enzymatically, although the degree of degradation was relatively low. However, such degradation was markedly accelerated by

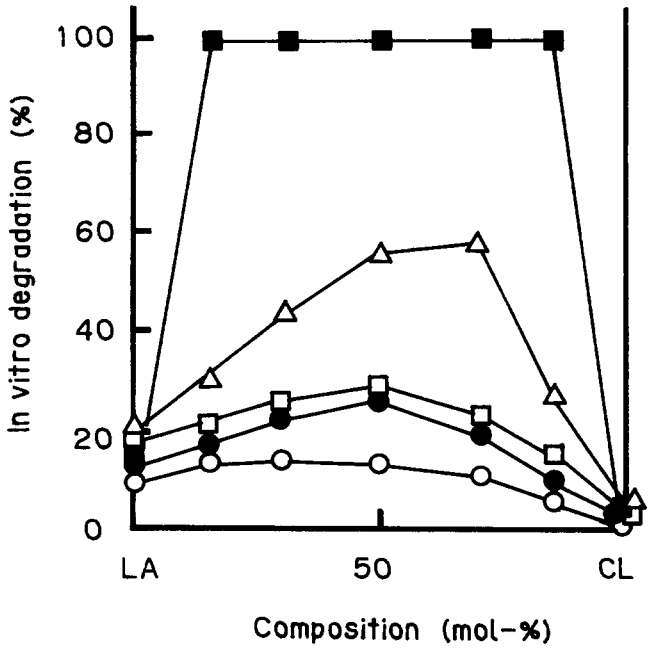

Figure 10 Effect of monomer composition on the *in vitro* degradation of copoly(LA/CL) with $\bar{M}_n = 2500$, treated at 37°C for 3 weeks in M/15 phosphate buffer solution (pH 7.2) containing (■) lipase from *Rhizopus delemer*, (△) lipase from hog pancreas, (□) carboxylic esterase from porcine liver, (●) lipase from wheat germ and (○) no enzyme (control)

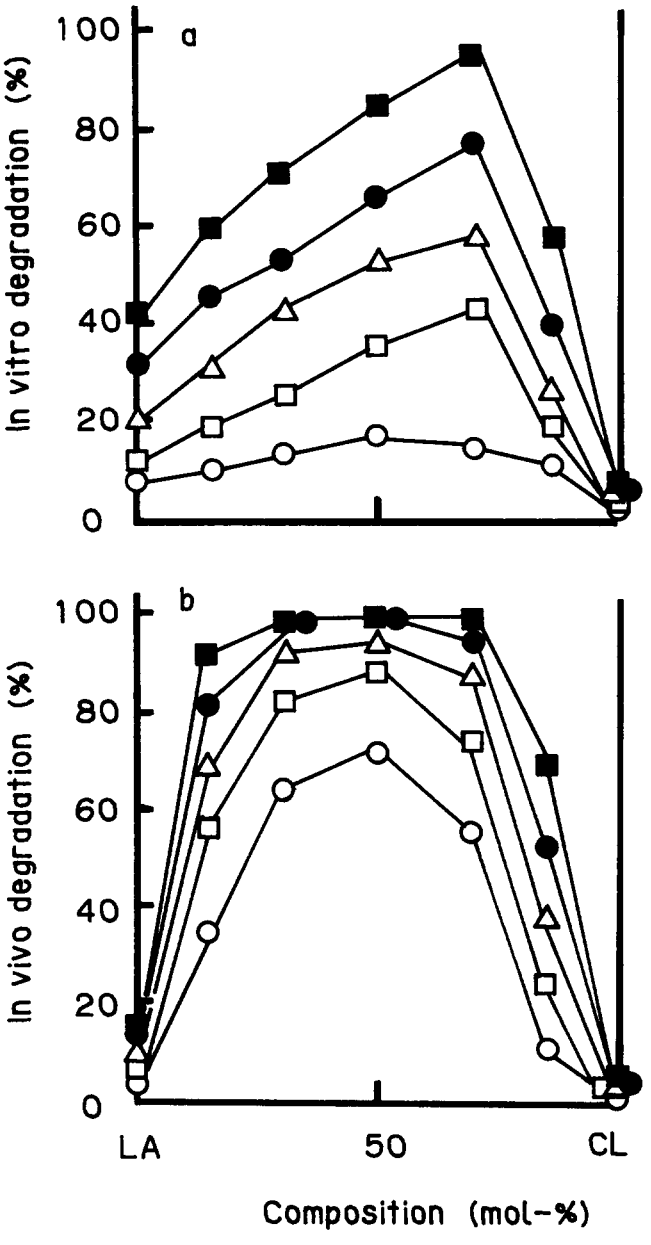

Figure 11 Effects of monomer composition on the *in vitro* and *in vivo* degradations of copoly(LA/CL) with $\bar{M}_n = 2500$. (a) *In vitro* tests carried out at 37°C in M/15 phosphate buffer solution (pH 7.2) containing lipase from hog pancreas. (b) *In vivo* tests performed by subcutaneous implantation in the back of rats. Tests carried out over periods of (○) 1 week, (□) 2 weeks, (△) 3 weeks, (●) 4 weeks and (■) 5 weeks

adding an esterase-type enzyme, and depends strongly on enzyme origin (lipases from wheat germ, hog pancreas and *Rhizopus delemer*) and kind of enzyme (carboxylic esterase or lipase). Of these, *Rhizopus delemer* lipase showed the strongest degradation activity, resulting in 100% degradation of copolymers ranging in composition from 15 to 85 mol% CL within 3 weeks. On the other hand, the changes in degree of *in vitro* degradation of copoly(LA/CL) with the passage of time were examined in a hog pancreas lipase-containing solution system, and the results are shown in *Figure 11a*. As can be seen, a highly crystallized waxy-type homopoly(CL) was scarcely degraded even after 5 weeks, in contrast to a moderate increase with time for a solid-type homopoly(LA). In a homopoly(CL) system, this is possibly due to the difficulty of penetrating enzyme into a highly

crystalline matrix. On the contrary, in the copolymer system, the *in vitro* degradation showed a marked acceleration with time and, as a result, reached a maximum at 30/70 mol% LA/CL copolymer system, degrading 95%. This is because it is easy for enzyme to penetrate into the pasty-type copolymer with amorphous structure owing to high swelling, in which the ester bonds located inside and outside of the matrix may be hydrolysed at the same time.

For comparison, the *in vivo* degradation of copoly-(LA/CL) was done by subcutaneous implantation in the back of rats, as seen clearly in *Figure 11b*. In the initial stage of implantation, the *in vivo* degradation gave a maximal value at 50/50 mol% LA/CL copolymer system. Finally, after 5 weeks implantation 100% degradation was observed on copolymers ranging in composition from 30 to 70 mol% CL, which are pasty. These findings mean that the amorphous and pasty copolymers are particularly subject to hydrolysis by the action of enzymes.

REFERENCES

1 Lundberg, R. D., Koleske, J. V. and Wischmann, K. B. *J. Polym. Sci.* 1969, **7**, 2915
2 Ito, K., Hashizuka, Y. and Yamashita, Y. *Macromolecules* 1977, **10**, 821
3 Lyudvig, Ye. B., Belen'kaya, B. G., Barskaya, J. G., Khomyakov, A. K. and Bogomolova, T. B. *Acta Polym.* 1983, **34**, 754
4 Hofman, A., Slomkowski, S. and Penczek, S. *Makromol. Chem., Rapid Commun.* 1987, **8**, 387
5 Endo, M., Aida, T. and Onoue, S. *Macromolecules* 1987, **20**, 2982
6 Kricheldorf, H. R., Jonte, J. M. and Dunsing, R. *Makromol. Chem.* 1986, **187**, 771
7 Kricheldorf, H. R. and Sumbel, M.-V. *Makromol Chem.* 1988, **189**, 317
8 Kricheldorf, H. R., Berl, M. and Scharnagl, N. *Macromolecules* 1988, **21**, 286
9 Schwope, A. D., Wise, D. L., Sell, K. W., Dressler, D. P. and Skornick, W. A. *J. Biomed. Mater. Res.* 1977, **11**, 489
10 Pitt, C. G., Gratzl, M. M., Jeffcoat, A. R., Zweidinger, R. and Schindler, A. *J. Pharm. Sci.* 1979, **68**, 1534
11 Pitt, C. G., Gratzl, M. M., Kimmel, G. L., Surles, J. and Schindler, A. *Biomaterials* 1981, **2**, 215
12 Woodward, S. C., Brewer, P. S., Moatamed, F., Schindler, A. and Pitt, C. G. *J. Biomed. Mater. Res.* 1985, **19**, 437
13 Pitt, C. G., Jeffcoat, A. R., Zweidinger, R. A. and Schindler, A. *J. Biomed. Mater. Res.* 1979, **13**, 497
14 Asano, M., Yoshida, M., Kaetsu, I., Imai, K., Mashimo, T., Yuasa, H., Yamanaka, H., Suzuki, K. and Yamazaki, I. *Makromol. Chem., Rapid Commun.* 1985, **6**, 509
15 Fukuzaki, H., Yoshida, M., Asano, M. and Kumakura, M. *Eur. Polym. J.* 1989, **25**, 1019
16 Asano, M., Fukuzaki, H., Yoshida, M., Kumakura, M., Mashimo, T., Yuasa, H., Imai, K., Yamanaka, H. and Suzuku, K. *J. Controlled Release* 1989, **9**, 111
17 Fukuzaki, H., Yoshida, M., Asano, M., Aiba, Y. and Kaetsu, I. *Eur. Polym. J.* 1988, **24**, 1029
18 Fukuzaki, H., Yoshida, M., Asano, M., Kumakura, M., Mashimo, T., Yuasa, H., Imai, K., Yamanaka, H., Kawaharada, U. and Suzuki, K. *J. Controlled Release* 1989, **10**, 293
19 Fukuzaki, H., Aiba, Y., Yoshida, M., Asano, M. and Kumamura, M. *Makromol. Chem.* 1989, **190**, 1553
20 Fukuzaki, H., Yoshida, M., Asano, M., Aiba, Y. and Kumakura, M. *Eur. Polym. J.* 1990, **26**, 457
21 Gilding, D. K., Reed, A. M. and Askill, I. N. *Polymer* 1981, **22**, 505
22 Asano, M., Yoshida, M., Kaetsu, I., Yamanaka, H., Nakai, K., Yuasa, H., Shida, K. and Oya, M. *J. Macromol. Sci.–Chem. (A)* 1984, **21**, 561
23 Kricheldorf, H. R., Mang, T. and Jonte, J. M. *Macromolecules* 1984, **17**, 2173
24 Crescenzi, V., Manzini, G., Calzolari, G. and Borri, C. *Eur. Polym. J.* 1972, **8**, 449
25 Tokiwa, Y. and Suzuki, T. *Nature* 1977, **270**, 76
26 Tokiwa, Y., Suzuki, T. and Takeda, K. *Agric. Biol. Chem.* 1986, **50**, 1323
27 Fields, R. D., Rodriguez, F. and Finn, R. K. *J. Appl. Polym. Sci.* 1974, **18**, 3571