Synthesis and Properties of Cyclic Oligo(Lactic Acid)

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Contents

Chapter 1

General Introduction

1.1 Background of the work

Cyclic molecules are completely different from linear molecules; they have neither a beginning nor an end. For example, large cyclic lactones, also called macrolides, which comprise >12 carbon atoms, show a variety of pharmacological properties such as the inhibition of bacterial protein production; however, linear lactones do not exhibit such properties [1–3]. Moreover, some cyclic molecules include other species inside their rings. For instance, it is well known that crown ethers include various alkali metals [4–6] and many cyclic polyamines and polyketones are known to form inclusion complexes [7–10] due to attraction of guest ions by their carbonyl groups. For example, cyclodextrin, which forms inclusion complexes with many molecules, [11–14] is used in food additives and ingredients [12]. The molecular encapsulation of food additives with cyclodextrin improves their stability. It is also well known that cyclodextrins include polymers such as poly(ethylene glycol) that form polyrotaxane. Using NMR and UV-Vis spectroscopies, Harada *et al*. discovered that *α*,*ω*-diaminopoly(ethylene glycol) and *α*-cyclodextrins form complexes and showed that a poly(ethylene glycol) chain is included in the cavities of α-cyclodextrins [11, 15, 16]. Furthermore, the specific structure of polyrotaxane is expected to be used for so-called "slide-ring materials" and is now used in practical applications [17, 18]. These distinct behaviors are not observed for linear molecules.

Cyclic polymers or oligomers, which are types of cyclic molecules, sometimes display different properties from linear polymers or oligomers. Weil *et al*. demonstrated the difference in viscosity between cyclic and linear poly(lactic acid) (PLA) [19]; moreover, their solubilities are different. Takano *et al*. studied the second virial coefficients and theta

temperatures of linear and cyclic polystyrene in cyclohexane [20] which indicates a difference in their solubilities in the solvent. Originally, the molecular structures of cyclic oligomers are highly deformed and have such a high ring strain energy that ring-opening can easily occur as observed for compounds such as PLA, and polycaprolactone [21]. And the ring-opening polymerization of cyclic poly(butylene terephthalate) (PBT) is an entropically driven polymerization [22]. Especially, polyesters can easily contain cyclic polymers or oligomers in the product that arises from the polymerization mechanism [23−25].

Thus, it is clear that cyclic polymers have many properties that are specific to their cyclic form. Syntheses and investigations of pure cyclic compounds have led to an understanding of their specific character. In this article, studies of synthesis, extraction, and functions of cyclic polyesters are reviewed.

1.2 Synthesis of cyclic oligoesters

Cyclic polyesters are generally obtained either by extraction as a by-product contained in the polymerized product obtained via direct polycondensation or by synthesis directly from an ester monomer using an appropriate catalyst.

1.2.1 Equilibrium

Most polyesters such as poly(ethylene terephthalate) (PET), PBT, PLA, and poly(butylene succinate) could be polymerized via direct polycondensation.

Regarding polymerization of polyesters, the Ruggli–Ziegler high dilution principle was developed. It indicates that high dilution favors the cyclization [26]. Stockmayer, and Kricheldorf summarized the equilibrium of polycondensation [23, 24, 27], which mentioned that the chain ends of the polycondensation including polyesters are in equilibrium with cyclic and linear polymers, and the amount of the cyclic oligomers is an increasing function of system volume. Kriecheldorf found that the theory could be applied to the production of

smaller cyclic oligomers at low conversion.

A rapid equilibration reaction, transesterification, and back-biting (Figure 1.1) are part of the polymerization, and reversible formation of cyclic monomers and oligomers occurs. Chain–chain, ring–chain, and ring–ring equilibria are found in this system and according to the law of self-dilution, an increasing number of cycles will be formed with increasing conversion such that, at conversion beyond 99%, the ring–ring equilibrium will dominate the thermodynamic parameters. Thus, a certain amount of cyclic compounds are produced via direct polycondensation of polyesters.

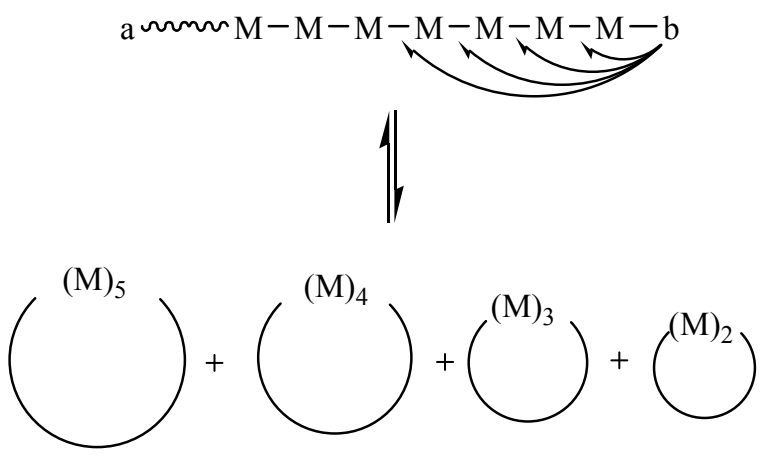

Figure 1.1 Ring–chain equilibration via ‘‘back-biting’’ [23].

In particular, Kéki *et al*. investigated the ring-chain equilibrium of melt polycondensation of D,L-lactic acid using MALDI-MS [28]. Poly(D,L-lactic acid)s polymerized at different temperatures were investigated using MALDI-MS. Both linear and cyclic oligomers were formed at 200°C (Figure 1.2), and only linear oligomers were formed at low temperature. The equilibrium constant K_x (eq 1, 2) was calculated from the peak intensities of linear and cyclic oligomers, and K_x-x plots were obtained (Figure 1.3), where x is the number of the repeating unit, and C_x is the x-mer linear chain and R_x is the x-mer ring.

Examination of the plots show that K_x values decrease with the extent of polymerization and increase with a rise in temperature. Notably, it is assumed that few cyclic oligomers were produced when the polymerization temperature was <150°C.

$$C_x + C_y \overset{K_x}{\rightleftharpoons} C_{x+y} + H_2O \qquad \text{(eq 1)}$$

$$C_{x+y} \overset{K_x}{\rightleftharpoons} R_x + C_y \qquad \text{(eq 2)}$$

As mentioned above, polyester contains cyclic compounds due to the equilibrium, which sometimes have a negative effect on their properties [29, 30]. Vermylen *et al*. reported that an equilibrium exists between PET and cyclic oligomers. PET contains a high amount of cyclic trimers which degrade the properties of film, fiber, and molding because the oligomers in PET migrate to the surface and crystalize.

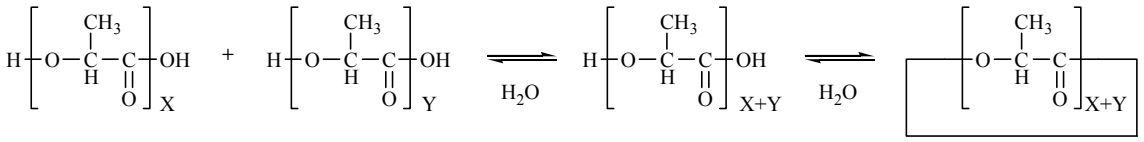

Figure 1.2 The formation of linear and cyclic oligo(lactic acid) [28].

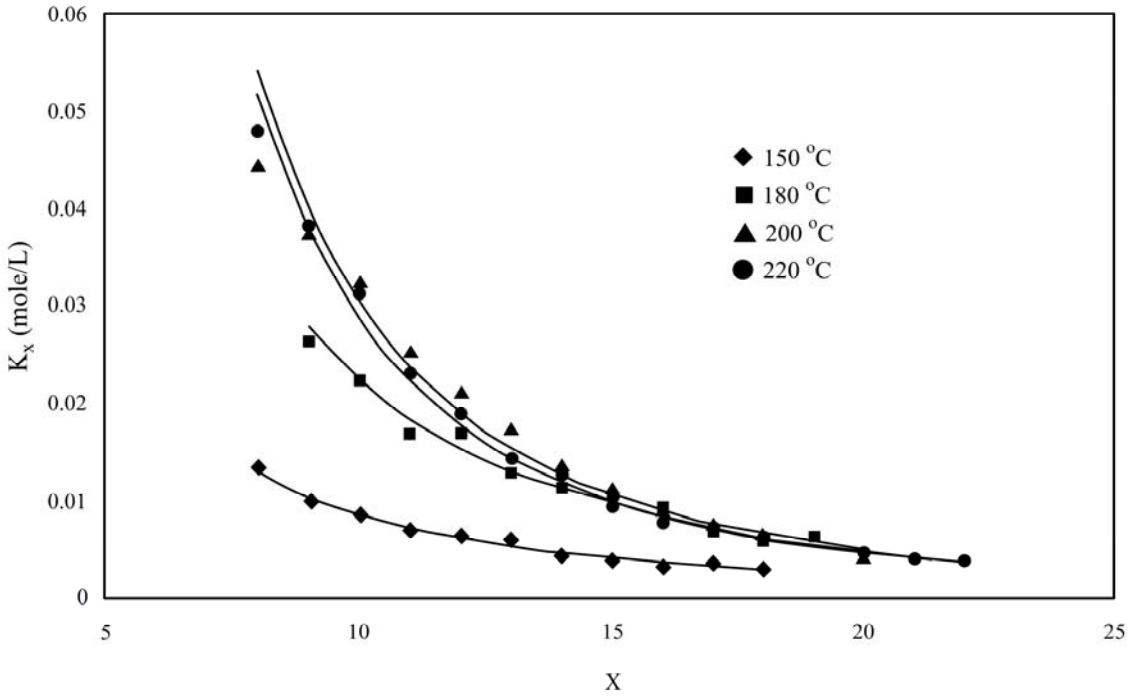

Figure 1.3 K_x-x plots in direct polycondensation PLA [28].

1.2.2 Extraction of cyclic oligoesters

The method of extracting oligomer is useful for the efficient utilization of by-products even though there is a small amount of oligomers in the polycondensation polymer.

One of the most popular ways of extracting cyclic polymers or oligomers only from polyesters is by exploiting their lack of an end group. Zhang *et al*. obtained cyclic poly(3-hydroxypropionic acid) (P[3-HP]) from a mixture of linear and cyclic P[3-HP] by removing linear esters by converting to their sodium salt and washing with saturated $NaHCO_3$ aqueous solution [31].

The author synthesized poly(L-lactic acid) (PLLA) via direct polycondensation of L-lactic acid at 160°C. Additionally, only the cyclic oligo(L-lactic acid) (c-OLLA) was extracted using organic solvents such as hexane and cyclohexane at 4°C; this was confirmed using proton nuclear magnetic resonance (^{1}H NMR) and electrospray ionization mass

spectrometry (ESI-MS) (Figure 2.2). This will be shown in detail in Chapter 2. The product extracted with hexane contained more cyclic oligomer, but the products extracted with methanol and hexane at room temperature contained both linear and cyclic oligomer. And the product extracted with cold hexane at 4°C contained only cyclic oligomer but linear one, which indicates that the cold hexane enabled to extract and isolate cyclic oligomer. The advantages of this method are that it is very simple and that the extraction residue can be recycled. The results indicated that the differences in the hydrophobicity, topology, and temperature dependence of the solubility of the PLLA between cyclic and linear oligomers enabled the selective extraction. In the study, the yield of cyclic oligomer was very small. The yield will be increased if polymerization was conducted in solution but bulk on the basis of Ruggli–Ziegler high dilution principle.

Cyclic oligomers of PET are also well-researched [29, 30, 32, 33]. Vermylen *et al*. and Lim *et al*. extracted and isolated oligomers using appropriate solvents such as methanol and 1,4-dioxane for PET and PET-poly(ethylene isophthalate) copolymer [29, 30]. To the best of our knowledge, in the case of PET, the separation of cyclic and linear oligomers was not reported because only a small amount of linear oligomer was present in the high molecular weight PET. Additionally, crystallization of cyclic PET oligomers is so easy that cyclic oligomers could be separated by recrystallization.

1.2.3 Synthesis of cyclic oligoesters and polyesters

Shin *et al*. synthesized cyclic PLA (c-PLA) from lactide using the N-heterocyclic carbene,1,3-dimesitylimidazol-2-ylidene (IMes) catalyst [34, 35]. Both cyclic and linear PLA with number-average molecular weights (M_n) of 15,000–30,000 were produced via ring-opening polymerization of L- and D-lactide. Benzyl alcohol was added as an initiator only for the synthesis of the linear PLA (Figure 1.4). Weil *et al*. also polymerized c-PLA of over 4,000 g/mol from lactide using the alumatrane-inspired catalyst that can be used actively

both in solution and melt (Figure 1.5) [19]. While these methods gave well-controlled polymerization, high conversion, and high molecular weight, they are not suitable for production of low molecular weight oligomers.

Bielawski and Kricheldorf synthesized cyclic polymers by ring-expansion polymerization using cyclic Ru carbenes and cyclic tin(IV) initiators [36, 37].

Piedra–Arroni *et al*. has found the synthesis method using dual systems combining $Zn(C_6F_5)_2$ with an organic base (an amine or a phosphine) [38]. The system catalyzes the ring-opening polymerization, which enables to produce cyclic block copolymer of polycaprolactone and polylactide. The M_w of the product polymers were above 5,000.

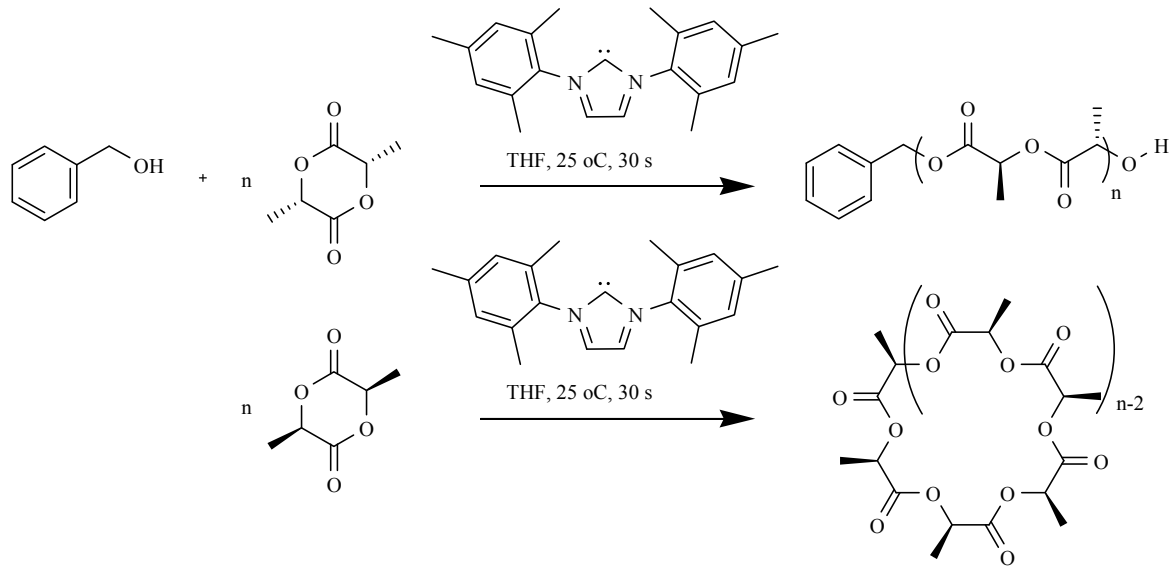

Figure 1.4 Polymerization of lactide catalyzed by IMes using different initiator [35].

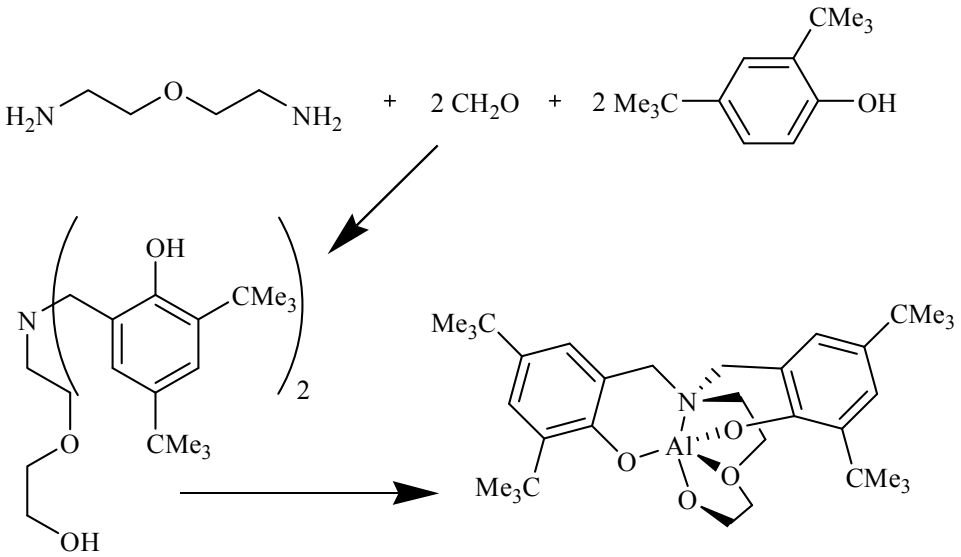

Figure 1.5 Alumatrane-inspired catalysis for synthesis of c-PLA [19].

Approaches for the synthesis of cyclic oligomers with 3–20 repeat units were also investigated. Chisholm *et al.* used PS-$C_6H_4CH_2NHLi(BuLi)_x$ as a catalyst to produce cyclic oligomers [39] from lactide. The catalyst includes the polystyrene (PS) chain to avoid bimolecular reactions. The obtained MALDI-MS spectrum is shown in Figure 1.6 and indicates the presence of cyclic oligomers with 9–20 repeat units. The presence of oligomers with M_n = 500–1,000 g/mol was confirmed by gel permeation chromatography (GPC).

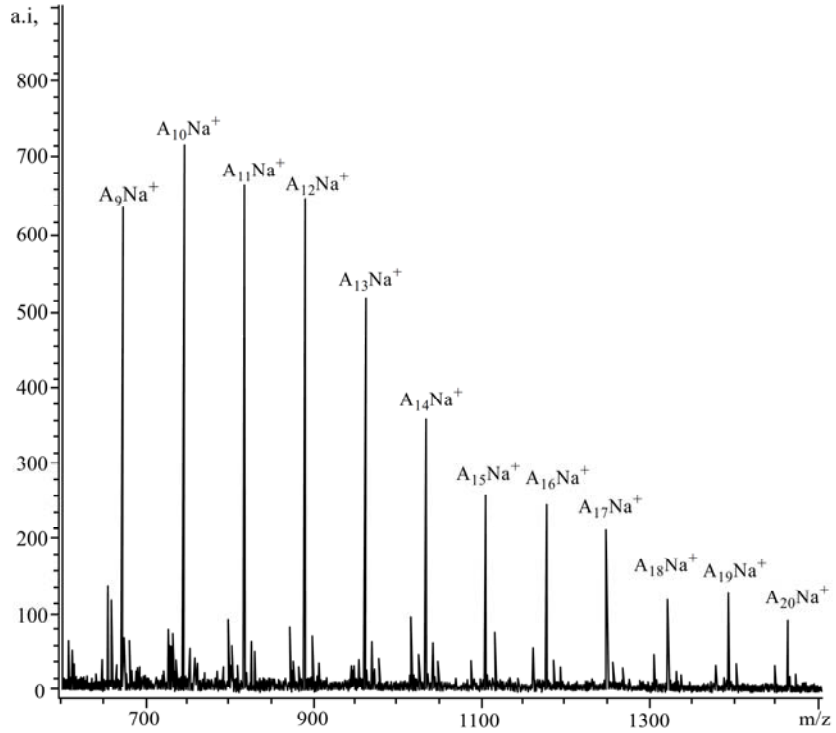

Figure 1.6 MALDI-MS spectrum of cyclic oligomer derived from PS-$C_6H_4CH_2NHLi(BuLi)_{3.7}$ (A = $C_3H_4O_2$) [39].

Click reaction is useful way to obtain cyclic oligoesters and polyesters. The copper-catalyzed Huisgen dipolar cycloaddition of a terminal azide and an alkynyl (Cu(I)-catalyzed azide−alkyne cycloaddition (CuAAc)) to form a 1,4-disubstituted 1,2,3-triazole enables the cyclization of linear polyesters to form cyclic polyesters. [40]. Ring closing enyne metathesis reaction is also used for forming cyclic molecules. Xie *et al*. synthesized poly(ε-caprolactone) (PCL) via the reaction using telechelic PCL [41].

Brunelle *et al*. reported that cyclic oligo(butylene terephthalate) was synthesized using isophthaloyl and terephthaloyl chlorides and aliphatic diols as monomers and amines as a catalyst [42]. Additionally, it was found that sterically-unhindered amines such as diazabicyclo[2.2.2]octane or quinuclidine accelerate the reaction (Figure 1.7).

In additional to these, various synthetic strategies of cyclic polyesters are reviewed by Hoskins and Grayson [43].

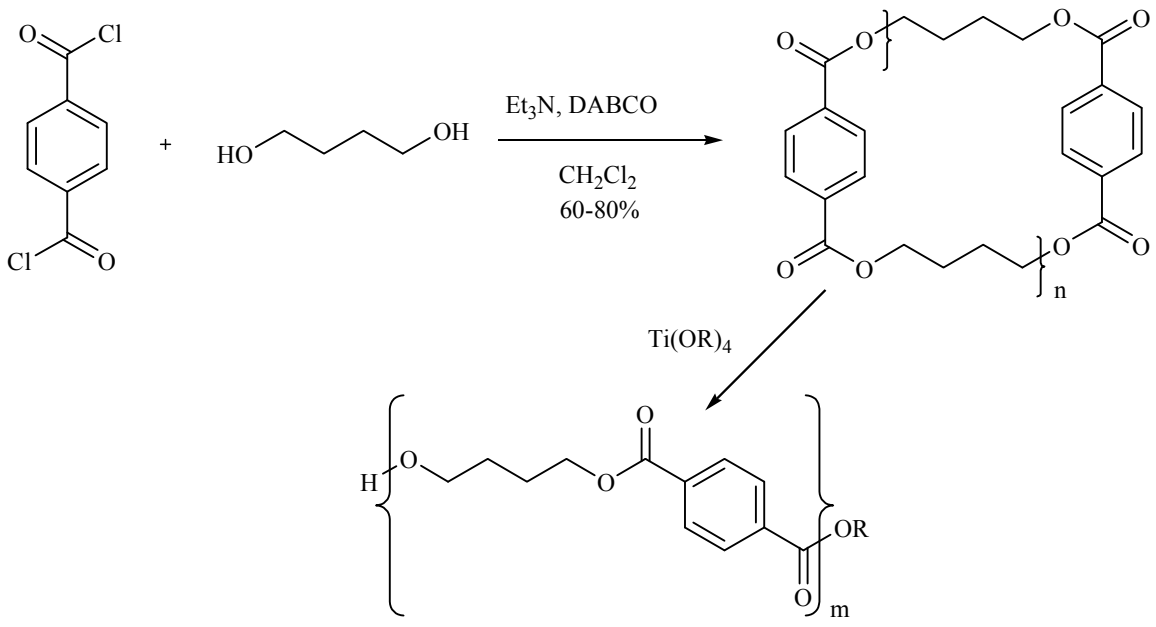

Figure 1.7 Preparation and polymerization of cyclic oligo(butylene terephthalate) [42].

1.3 Properties and functions of cyclic poly(lactic acid)s and oligo(lactic acid)s

1.3.1 Behavior of cyclic poly(lactic acid)

The cyclic compounds obtained using the various methods described in the previous section exhibit different characteristic behavior despite their similar degree of polymerization.

It is well known that linear PLLA and linear poly(D-lactic acid) (PDLA) form a stereocomplex structure during crystallization. Shin *et al*. showed that the combinations of "cyclic PLLA + linear PDLA" and "cyclic PLLA + cyclic PDLA" also form stereocomplexes [35]. Analysis of wide angle X-ray scattering (WAXS) and small angle X-ray scattering (SAXS) after annealing at 150°C indicated that while every combination formed a stereocomplex, their lamellar thickness and long period values were different (Figure 1.8, Table 1.1). For WAXS pattern (2)–(4) in Figure 1.8, the almost the same stereocomplex crystalline was formed, however, the lamellar thickness and long period of cyclic PLLA were larger than those of linear PLLA. Similarly, those of "cyclic PLLA + linear PDLA" and "cyclic PLLA + cyclic PDLA" were also larger than those of "cyclic PLLA + cyclic PDLA". This indicates that the formation of crystalline lamellae is more difficult for cyclic polymers than it is for linear polymers. Cao *et al*. also showed that the topology and entanglement of the molecular chain largely affect the thermal properties [44]. These results indicate that the difference in topology changed the crystal formation.

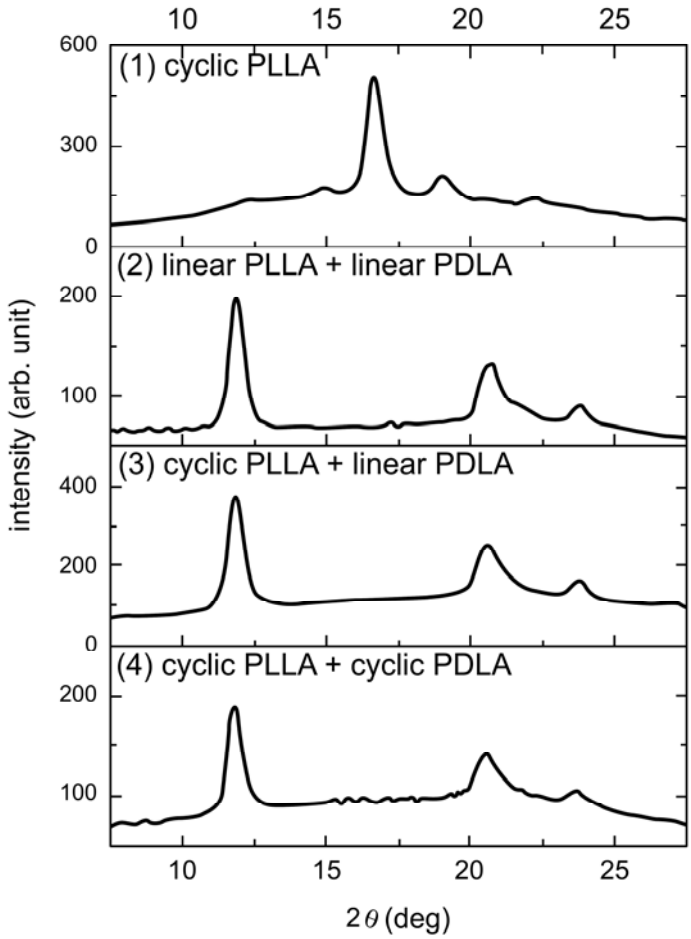

Figure 1.8 WAXS patterns after annealing at 150°C (90°C for cyclic PLLA) for 24 h [35].

Table 1.1 Lamellar thickness and long period of PLA including cyclic PLA [35]

Composition	Lamellar thickness (nm)	Long period (nm)
Linear PLLA	8	16
Cyclic PLLA	10	20
Linear PLLA + linear PDLA	7	14
Cyclic PLLA + linear PDLA	8	16
Cyclic PLLA + cyclic PDLA	8	15

The differences of the elution volume between cyclic and linear PLA were investigated directly and precisely by light scattering (LS) and GPC. Weil *et al*. synthesized numerous types of cyclic and linear PLA by using alumatrane-inspired catalyst (Figure 1.5) and bis(2-ethylhexanoate)-tin [19]. The absolute molecular weight and elution volume were obtained using LS and GPC, respectively. The plots of elution volume and absolute molecular weight indicated that the c-PLA exhibits a higher elution volume than the linear PLA (Figure 1.9). Furthermore, Mark–Houwink–Sakurada plots of cyclic and linear PLA suggested that the entanglement of the c-PLA is smaller than that of the linear PLA (Figure 1.10).

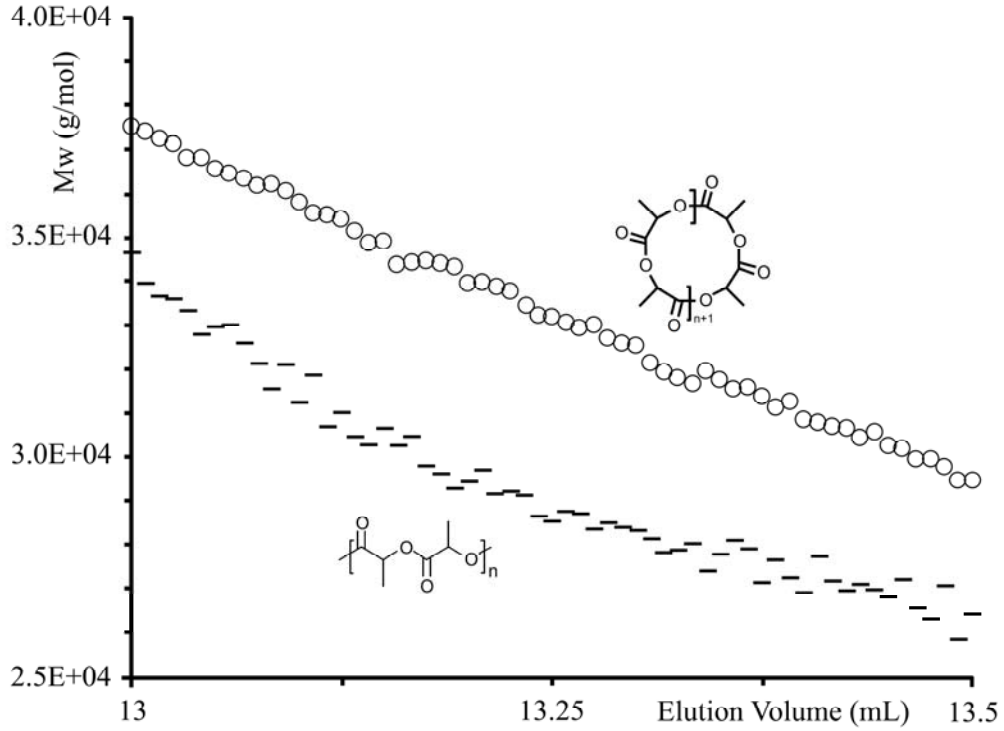

Figure 1.9 Molecular weight *vs*. elution volume for linear PLA (−) and the PLA produced by alumatrane-inspired catalyst (○) [19].

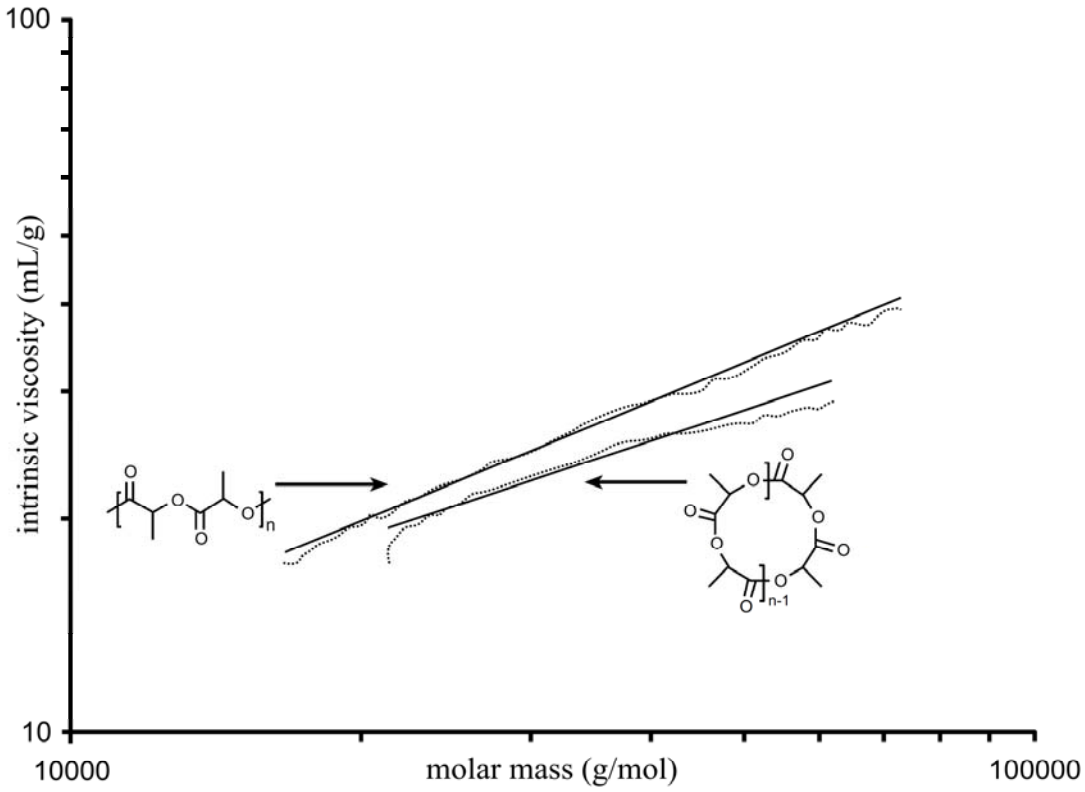

Figure 1.10 Mark–Houwink–Sakurada plots of cyclic and linear PLA [19].

1.3.2 Function and Properties of cyclic oligo(lactic acid)

Unlike the investigations of cyclic polymers described above, Chisholm *et al*. reported on the function of OLAs. Chisholm *et al*. investigated the complexation of c-OLA and sodium tetraphenylborate ($NaBPh_4$) [39]. The c-OLAs with many different numbers of cycles were synthesized using an appropriate catalyst, and it was assumed by Chisholm *et al*. that c-OLA with a certain number of cycles would be selectively removed in the presence of a guest ion. Then, the benzene solution of the mixture of c-OLAs was added to the $NaBPh_4$ and heated to 60°C. It was found that only the c-OLA with 6 repeat units (C_6) was selectively removed. The c-OLA probably includes the sodium ion in the cyclic ester cavities. In the formation of the C_6-sodium ion complex, it is assumed that the two C_6 rings bind to the sodium ion, and three ketonic oxygen atoms are directed toward the inside of the cavity, while

the other three are directed toward the outside of the cycle (Figure 1.11). These results strongly suggested that c-OLA forms an inclusion complex with alkali metal ions.

There are some interactions between c-OLLA and various alkali metal ions, which will be shown in detail in Chapter 3.

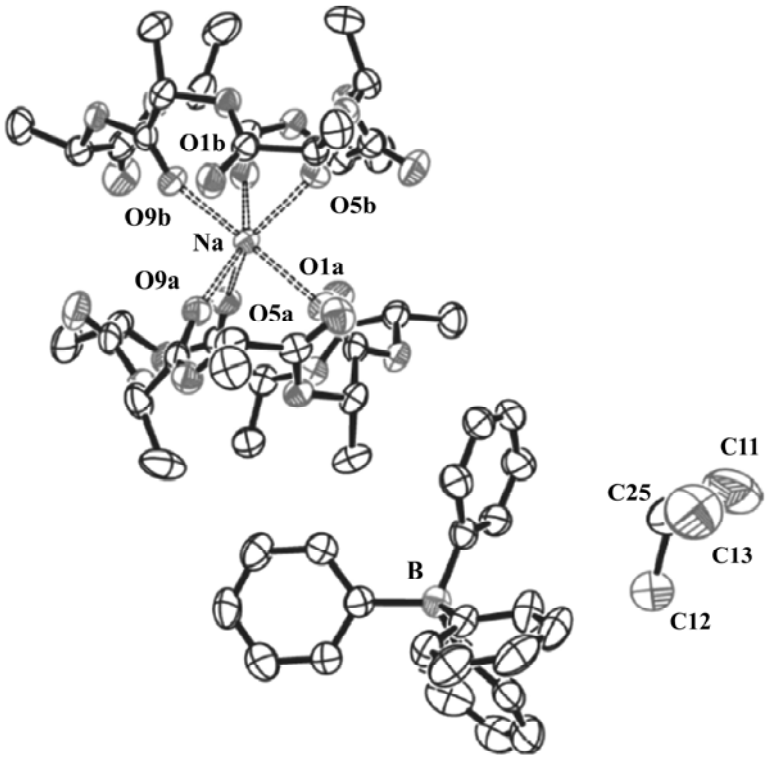

Figure 1.11 Estimated formation of the C_6-sodium ion complex [39].

1.3.3 Functions of cyclic oligo(lactic acid)

In medicine, c-OLLA is recognized as an antitumor material. Takada *et al*. and Aizawa *et al*. reported that c-OLLA suppresses the growth of tumor cells by inhibiting the glycolytic system [45, 46]. c-OLLA inhibited the activity of both pyruvate kinase and lactic hydrogenase, which are important enzymes for cell growth. Notably, the activity of lactic dehydrogenase was remarkably suppressed in the tumor cells relative to the normal cells (Figure 1.12).

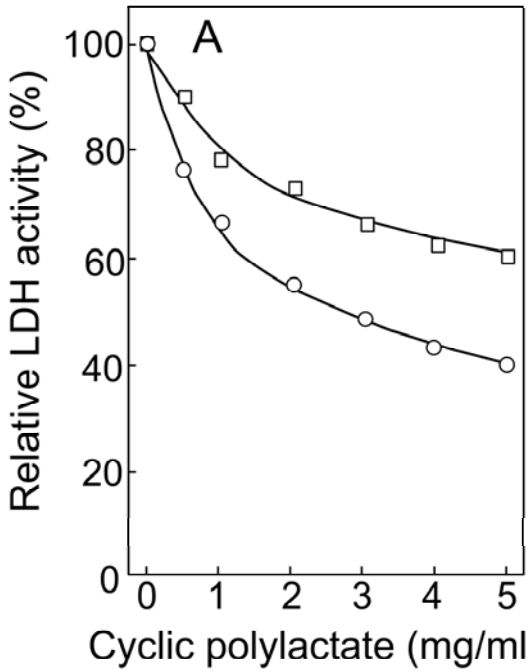

Figure 1.12 Effect of c-OLLA on the lactic hydrogenase activity from ascites tumor cells (○) and rabbit muscle (normal cell, □) [45].

Considering that cyclic oligomers with 14–16 repeat units are generally known as macrolides [1, 3, 47], c-OLLA also shows an effect on the enzymatic reaction or enzyme itself. It is possible that the formation of an inclusion complex of c-OLLA with ions as described above is related to the pharmacological effect.

As pointed in the article [43], one of the most possible applications is medical use. For example, owing to the ability of c-OLLA to transport more sodium ions than PLLA, the interaction could be utilized for ion transport [44]. Since c-OLLA derived from PLLA is biocompatible and biodegradable, it could be used as a drug delivery system with increased transport efficiency of particular ions to specific sites in human body.

It should also be mentioned that cyclic oligo(lactic acid) are used as plasticizer. Martin *et al*. showed the possibility of using oligo(lactic acid) as plasticizer in PLA. Adding 10% of OLA decreases modulus by 40% [48]. Furthermore, Anderson *et al*. studied about tuning the hydrolytic degradation rate of PLA by adding OLA as plasticizer [49].

Conclusion

The synthesis and function of cyclic polyesters and oligoesters were reviewed. Cyclic oligoesters are produced as byproducts in direct polycondensation, inhibit polymer growth, and degrade the properties of the polymer; however, synthetic oligomers provide high performance polymers when polymerized with appropriate catalysts. Additionally, unlike the linear oligoesters, cyclic oligoesters containing c-OLLA form inclusion complexes with alkali metal ions. It is possible that cyclic oligoesters transport ions selectively because the included ion depends on the size of its inner cavity. In medicine, the antitumor effect of c-OLLA may be related to such inclusion behavior. The development of facile methods for the production of fine cyclic esters will not only improve the properties of polymers, but will also accelerate the investigations and utilization of cyclic esters in medical applications.

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Chapter 2

Cyclic Oligolactic Acid in Direct Polycondensation PLLA and Its Extraction with Organic Solvent

2.1 Introduction

Poly(L-lactic acid) (PLLA) has attracted attention for its carbon neutrality and biodegradability. Various grades of PLLA, including some with molecular weights greater than one hundred thousand, are now industrially produced. Generally, these polymers are regarded as linear molecules and are synthesized by the ring-opening polymerization of L,L-lactide [1] or the direct condensation polymerization of L-lactic acid with catalyst [2]. On the other hand, methods for the synthesis of cyclic poly(L-lactic acid) (c-PLLA) have been reported recently. For example, c-PLLA with molecular weights of approximately 4,000–39,000 (the number of repeat units; 60–540) was synthesized using an alumatrane-inspired catalyst [3]. Waymouth *et al*. also prepared c-PLLA by the zwitterionic polymerization of lactide using the N-heterocyclic carbene 1,3-dimesitylimidazol-2-ylidene (IMes) as the catalyst and investigated the crystallinity of the polymer [4].

Cyclic esters, also known as lactones, are found in living systems. For example, 3-methyl-4-octanolide is present in oak trees [5], while exaltolide with a 16-membered ring is known as a musk perfume compound [6].

In the field of medicine, cyclic oligo esters with 14–16 repeat units are generally known as macrolides, which exhibit antibacterial activity [7–9]. In addition, cyclic oligo(L-lactic acid) (c-OLLA), which is composed of lactic acid, is recognized as an antitumor material, and studies to elucidate the antitumor activity of c-OLLA have been conducted [10, 11]. OLLA that includes both linear and cyclic compounds suppresses the growth of cancer cells *in vivo* by directly affecting the glycolytic system. However, it is not clear whether it is

the linear or cyclic compounds that affect the cancer cells, because there is no description for the preparation of OLLA which does not include linear OLLA (l-OLLA). Therefore, in order to elucidate the function of the cyclic compounds, the isolation of pure c-OLLA is necessary.

Cyclic compounds are assumed to be contained in the products obtained from the direct polycondensation of lactic acid, the author attempted to isolate cyclic compounds simpler way, and study the interaction behavior of cyclic compounds contained in PLLA prepared by direct melt polycondensation.

2.2 Experiment

2.2.1 Materials

L-lactic acid (90 wt% aq. solution, HiPure 90, Purac Biochem. bv., NLD) was used without purification. Special grade chloroform, methanol, diethyl ether, hexane, and cyclohexane (Sigma-Aldrich Inc., USA) solvents were also used without purification.

2.2.2 Polycondensation of L-lactic acid

PLLA was synthesized by the direct condensation polymerization method reported in reference [12], except that no catalyst was added. A 300-ml three-neck separable flask was equipped with a magnetic stirrer and reflux condenser, which was connected to a vacuum system through a cold trap. First, L-lactic acid (100 g) was charged into the flask and heated to 160°C in stages with stirring while the pressure was reduced stepwise to 4 kPa, at which point, the reaction was continued for 21 h. As the reaction proceeded, the solution gradually became viscous. When the reaction was complete, the flask was cooled to room temperature (26°C), and the product was crushed into a powder using a mortar.

2.2.3 Extraction of cyclic oligo(lactic acid)

Powdered PLLA (5 g) was added to a solvent (50 ml), including methanol, diethyl ether, hexane, and cyclohexane, at room temperature or 4°C (ice water bath). PLLA was not completely dissolved in any of the solvents. Insoluble materials were removed from the mixture by filtration. The filtrates were then analyzed by electrospray ionization mass spectrometry (ESI-MS), nuclear magnetic resonance (NMR) spectroscopy, and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) after solvent removal under vacuum at 40°C.

2.2.4 Determination of molecular weight

Each sample was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) containing 10 mM trifluoroacetic acid (0.68 g/L) in order to determine its weight-average molecular weight (M_w) and number-average molecular weight (M_n) by gel permeation chromatography (GPC, e2695, Waters Co., USA) using a refractive index (RI) detector. A column for organic solvents (Shodex HFIP-806M, Showa Denko k.k., JPN) was used as the guard column. The operating conditions were as follows: column temperature, 30°C; flow rate, 1 ml/min; calibration, polystyrene.

2.2.5 Determination of optical purity

The optical purity of the lactic acid units was determined by high performance liquid chromatography (HPLC, LC-20 series, Shimadzu Co., JPN) of the lactic acid formed after the acid hydrolysis of the polymer samples. A Mitsubishi Chemical MCI GELCRS10W column was used for isolation of the optical isomers. Before the analysis, a 1.5 mg of polymer sample was dipped in 0.5 ml of 2 M NaOH for hydrolysis at room temperature. The resultant solution was then neutralized with 1 M H_2SO_4 (0.5 ml), diluted by 10%, and injected to the analyzer. The analytical conditions are as follows: column temperature, 35°C; solvent for elution, 1

mM $CuSO_4$ aqueous solution; and flow rate, 0.5 ml/min. Both L-and D-lactic acids were detected by a UV detector at wavelength 254 nm. Optical purity was evaluated by the following %ee:

$$\%ee = 100 \times ([L] - [D])/([L] + [D])$$

where [L] and [D] denote the molarities of L- and D-lactic acids, respectively.

2.2.6 Mass spectrometry of extracted products

ESI-MS spectra were recorded using an ion trap mass spectrometer (amaZon SL, Bruker Daltonics Inc., USA). The operating conditions were as follows: flow rate, 240 µl/h; capillary temperature, 175°C; spray voltage, 4.5 kV; and capillary voltage, 40 V. Samples were dissolved in liquid chromatography/mass spectrometry (LC/MS)-grade acetonitrile (Kanto Chemical Co., JPN).

2.2.7 NMR analysis

^{1}H NMR spectra were recorded using an AVANCE Series 300 MHz spectrometer (Bruker BioSpin Co., USA) operating at 300 MHz. Samples were dissolved in DMSO-d_6 (Sigma-Aldrich Inc.) containing 0.03 vol% tetramethylsilane as the internal reference. The sample concentration was 100 mg/ml, and the spectra were recorded at 23°C.

2.2.8 Thermal analysis

Melting and glass transition temperatures (T_m and T_g, respectively) were determined by differential scanning calorimetry (DSC, Q200, TA Instruments, USA). Samples were heated at a rate of 5°C/min under a flow of nitrogen gas.

2.2.9 Mass spectrometry of polymers and extracted products

MALDI-MS spectra were recorded using an autoflex speed-KF system (Bruker

Daltonics Inc.) equipped with a nitrogen laser (Smartbeam-II, 355 nm). The acceleration voltage was 19 kV, and the spectra were obtained in the linear, positive ion mode with pulsed ion extraction (120 ns). The energy of the laser beam was set at approximately 70%, and the spectra were acquired in m/z range 500−10,000. External calibration was performed using appropriate samples of angiotensin II. The samples were measured by employing in a solvent-free MALDI-MS method in trans-3-indoleacrylic acid matrix in the presence of NaI as cationization agent.

2.3 Results and Discussion

2.3.1 Assignment of poly(L-lactic acid) and extracted products

The M_n and M_w of polymerized PLLA were 7,500 and 9,200, respectively. The optical purity of the PLLA was 96.0%ee while that of L-lactic acid was 99.9%ee. Figure 2.1(A) shows the ^{1}H NMR spectrum of PLLA. The signals were assigned on the basis of data reported in reference [13]. The signals were assigned as in Figure 2.1. The protons relatively close to end groups, assigned as signal (a_2), (a_3), and (a_4) tend to be affected by carboxyl end group or hydroxyl end group. Notably, the signal attributed to the proton closest to the carboxyl end group (a_2) in Figure 2.1(A) was detected in the spectrum of PLLA, indicating that it contained linear oligo(L-lactic acid). In addition, the ^{1}H NMR spectra of the products extracted with methanol at room temperature and hexane at 4°C are also shown in Figure 2.1(B) and Figure 2.1(C). For the product extracted with methanol, the signal attributed to the proton closest to the carboxyl end group was also observed, indicating that l-OLLA was extracted. This signal was also detected when methanol at 4°C or diethyl ether was used as the extraction solvent (data not shown). In contrast, no signal attributed to the proton closest to the carboxyl end group was observed in the ^{1}H NMR spectra of the products obtained after extraction with cold hexane or cyclohexane. Rather, the signal due to the proton farthest away

from the end group (a_1) in Figure 2.1(C) was detected; therefore, negligible amount of linear oligomer (l-OLLA) was present in these extracted products.

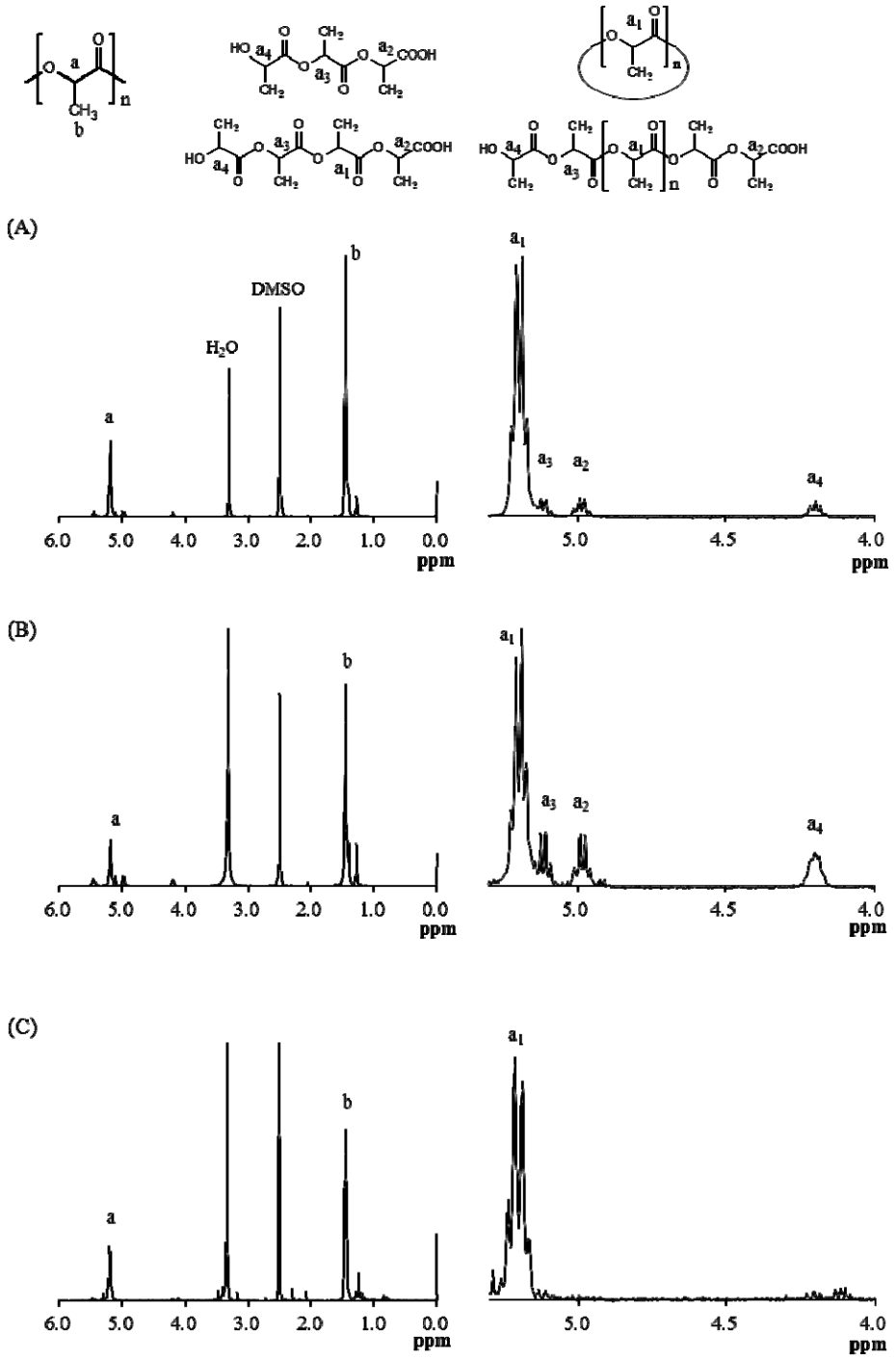

Figure 2.1 ^{1}H NMR spectra of (A) PLLA and (B) products extracted with methanol (r.t.), and (C) hexane (4°C).

2.3.2 Mass spectrometry of extracted products in solution

Figure 2.2 shows the ESI-MS spectra of the products extracted using each solvent. Notably, peaks for l-OLLA and c-OLLA adducted with Na^+ and K^+ were detected. The products extracted with methanol and diethyl ether at room temperature contained both l-OLLA and c-OLLA. It can be seen from the MS spectrum of the product extracted with methanol (Figure 2.2(A)) that OLLA with 3–20 repeat units was obtained. The yield of the methanol-extracted product was 6.79 wt%. While the product extracted with hexane at room temperature also contained both l-OLLA and c-OLLA (Figure 2.2(B)), the quantity of c-OLLA was much higher than that for the products extracted with methanol or diethyl ether. Furthermore, the product extracted with cold hexane at 4°C contained only c-OLLA, and no l-OLLA was detected (Figure 2.2(C)). Similar results were obtained when cyclohexane was used as the extraction solvent. The yield of the hexane-extracted product was 0.047 wt%, and the number of repeat units was 5–16.

The evaluation of the ESI-MS results for the product extracted with hexane at 4°C also indicated that there was appreciable interaction between c-OLLA and alkali metal ions in the acetonitrile solution because c-OLLA has no end group for binding to alkali metal ions. Though it cannot be a direct support of specific binding, it is assumed that the cavity of c-OLLA attracted and included the alkali metal ions.

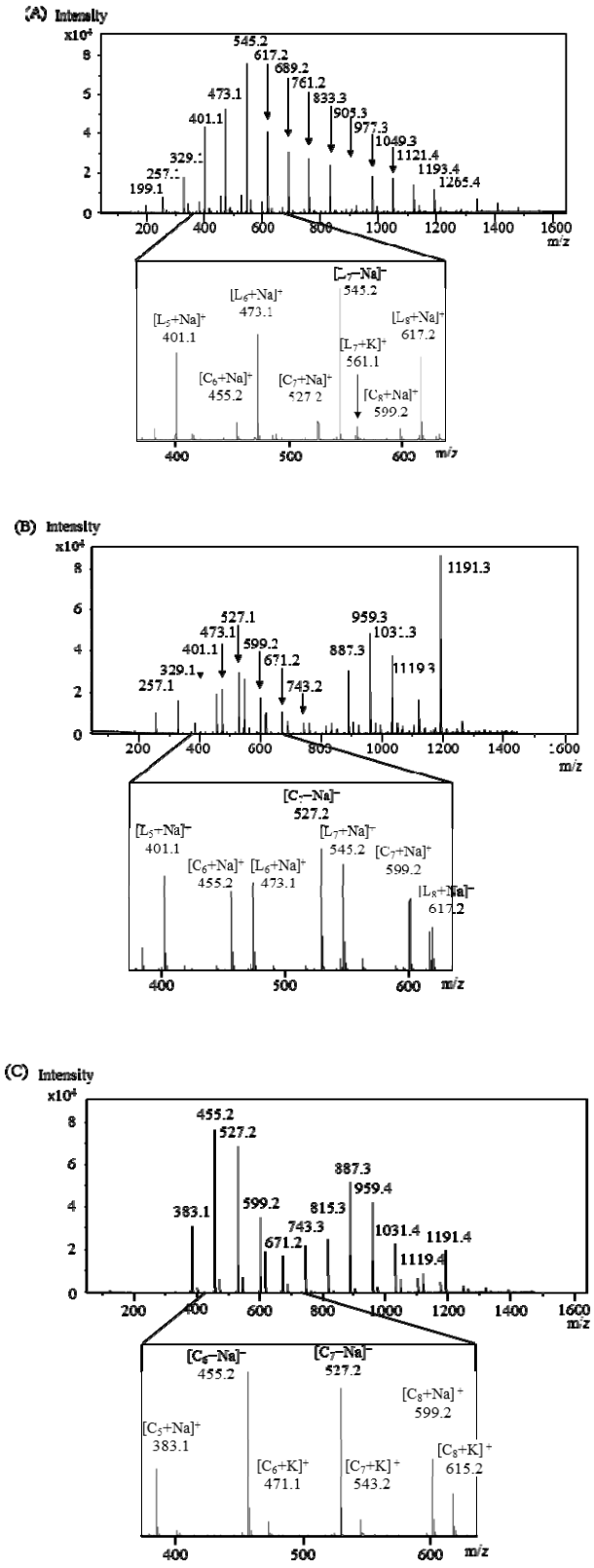

Figure 2.2 ESI-MS spectra of products extracted with (A) methanol (r.t.), (B) hexane (r.t.), and (C) hexane (4°C). $[L_5+Na]^+$ is due to the Na^+ adduct of l-OLLA with $n = 5$, and $[C_8+K]^+$ is due to the K^+ adduct of c-OLLA with $n = 8$.

Generally, cyclic compounds play a significant role in molecular recognition chemistry due to their structural characteristics. It is well known that some cyclic compounds such as crown ethers and cyclodextrins serve as host molecules, forming molecular inclusion complexes with specific guest molecules [14]. Crown ethers have been widely studied for many years and are used in many fields [15–18].

It is possible that the c-OLLA formed inclusion complexes with Na^+ and K^+ in a manner similar to crown ethers. Figure 2.3 shows the chemical structure of c-OLLA (n = 6) and crown ether. It should also be noted that c-OLLA may be a novel host molecule, unlike crown ethers, which was produced from biocompatible and biodegradable polymer that can be used in human body.

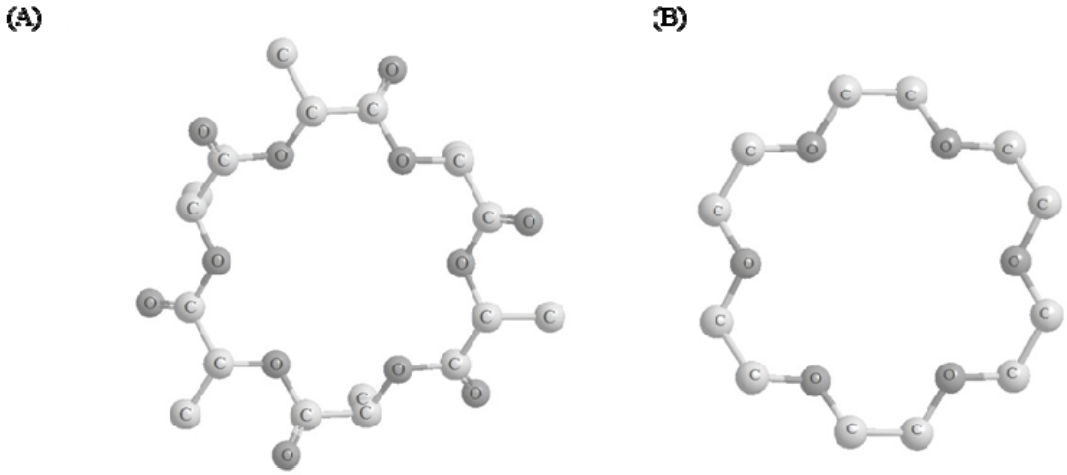

Figure 2.3 Ball-and-stick model of (A) c-OLLA (n = 6) and (B) crown ether (18-crown-6).

2.3.3 Thermal property of poly(L-lactic acid) and oligo(L-lactic acid)

Next, PLLA and extracted products were evaluated by DSC, and the results of 1st heating are presented in Figure 2.4. It can be seen in the figure that a melting peak was detected for both PLLA and the product extracted using cold methanol, while only a glass transition point was observed for the product extracted using cold hexane. This result indicates that PLLA and the product extracted with cold methanol which contains l-OLLA

mainly, have crystallinity, while the product extracted with cold hexane, more specifically, c-OLLA is amorphous. The glass transition temperatures (T_g) for PLLA and l-OLLA and c-OLLA were 52, 40, and 22°C, respectively. This fact indicates that molecular chain in c-OLLA was easy to move even though below T_g and it made it easy to be extracted with poor solvent like hexane as shown in Figure 2.2(C). In contrast, l-OLLA was also easy to move at room temperature (26°C), so that not only c-OLLA but also l-OLLA was extracted by hexane. The liquid-state linear and cyclic oligomers readily dissolved in various solvents near or above the T_g of PLLA, while the liquid state oligomer transformed to a solid (or glassy) state below the T_g, and as a result, its solubility decreased drastically.

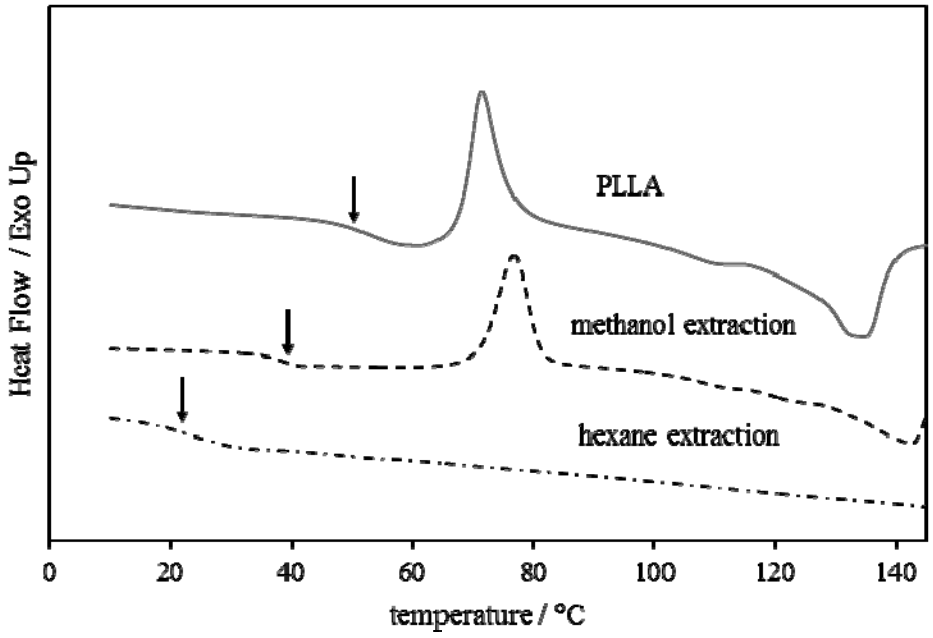

Figure 2.4 DSC results for PLLA and the products extracted with methanol and hexane at 4°C. T_g values are indicated by arrows.

2.3.4 Solubility of oligo(L-lactic acid) in solution

Furthermore, the solubility parameters (SPs) for hexane and cyclic oligo(L-lactic acid) were calculated by the van Krevelen method [19] and found to be 14.8 $MPa^{0.5}$ and 19.2 $MPa^{0.5}$, respectively, regardless of the number of repeat units. The SP of l-OLLA, on the other hand, is dependent on the number of repeat units; for example, the SPs of l-OLLA trimer and octamer are 22.6 $MPa^{0.5}$ and 20.5 $MPa^{0.5}$, respectively. In general, the difference in the SPs for hexane and c-OLLA is small, and therefore, the differences in the polarity of the solvents and topology of the oligomers likely influence the solubility of c-OLLA only at low temperatures. As a result, hexane and cyclohexane were only able to dissolve c-OLLA in a cold environment. Therefore, the differences in the polarity of the solvents likely influence the solubility of c-OLLA only at low temperatures. As a result, hexane and cyclohexane were only able to dissolve c-OLLA in a cold environment. In addition, the difference in the topology of the linear and cyclic molecules affects their solubility, because it is more difficult for cyclic molecules to aggregate.

It should be noted here that there are other methods for obtaining cyclic compounds, such as the removal of linear esters by conversion to their corresponding sodium salts [20]. While such an approach is an effective technique for obtaining cyclic compounds and requires rather complicated operation, solvent extraction as demonstrated in this study is a simpler method for extracting c-OLLA, and residues could be hydrolyzed into low molecular linear compounds. Then the c-OLLA could be produced again for higher yield.

2.3.5 Mass spectrometry of polymers and extracted products in bulk

The MALDI-MS spectra of PLLA and the product extracted using cold hexane were obtained to confirm the mass of each molecular, and are shown in Figure 2.5. Peaks for Na^+ adducted ions of both linear and cyclic products, $[M+Na]^+$ and $[M–H+2Na]^+$, which are often detected in MS measurement [21], were observed in the low-molecular-weight range (m/z =

500–1,500) in the spectrum of PLLA (Figure 2.5(A-1)). The cyclic products are indicated by the arrows in the Figure. On the other hand, no cyclic products were detected in the high-molecular-weight range (m/z > 2,000) in the spectra of the PLLA (Figure 2.5(A-2)). And products extracted from hexane at 4°C didn't contain linear products. These results indicate that no high-molecular-weight c-OLLA was produced in the direct melt condensation of lactic acid to form PLLA. It is thought that the concentration of PLLA terminating groups drastically decreases as high-molecular-weight linear products are generated during polymerization. As a result, the probability of the terminating groups colliding also decreases, and intramolecular esterification barely proceeds. Meanwhile, the low-molecular-weight linear molecules can readily cyclize. Furthermore, it is difficult to increase the molecular weight through the transesterification of the cyclized oligomers if no catalyst is present. This assumption was confirmed by the fact that an L-lactide that forms cyclic products analogous to the cyclic oligomers did not polymerize when heated at 180°C without the addition of a catalyst. That is, the c-OLLA may affect direct polycondensation and disturb molecular elongation of PLLA.

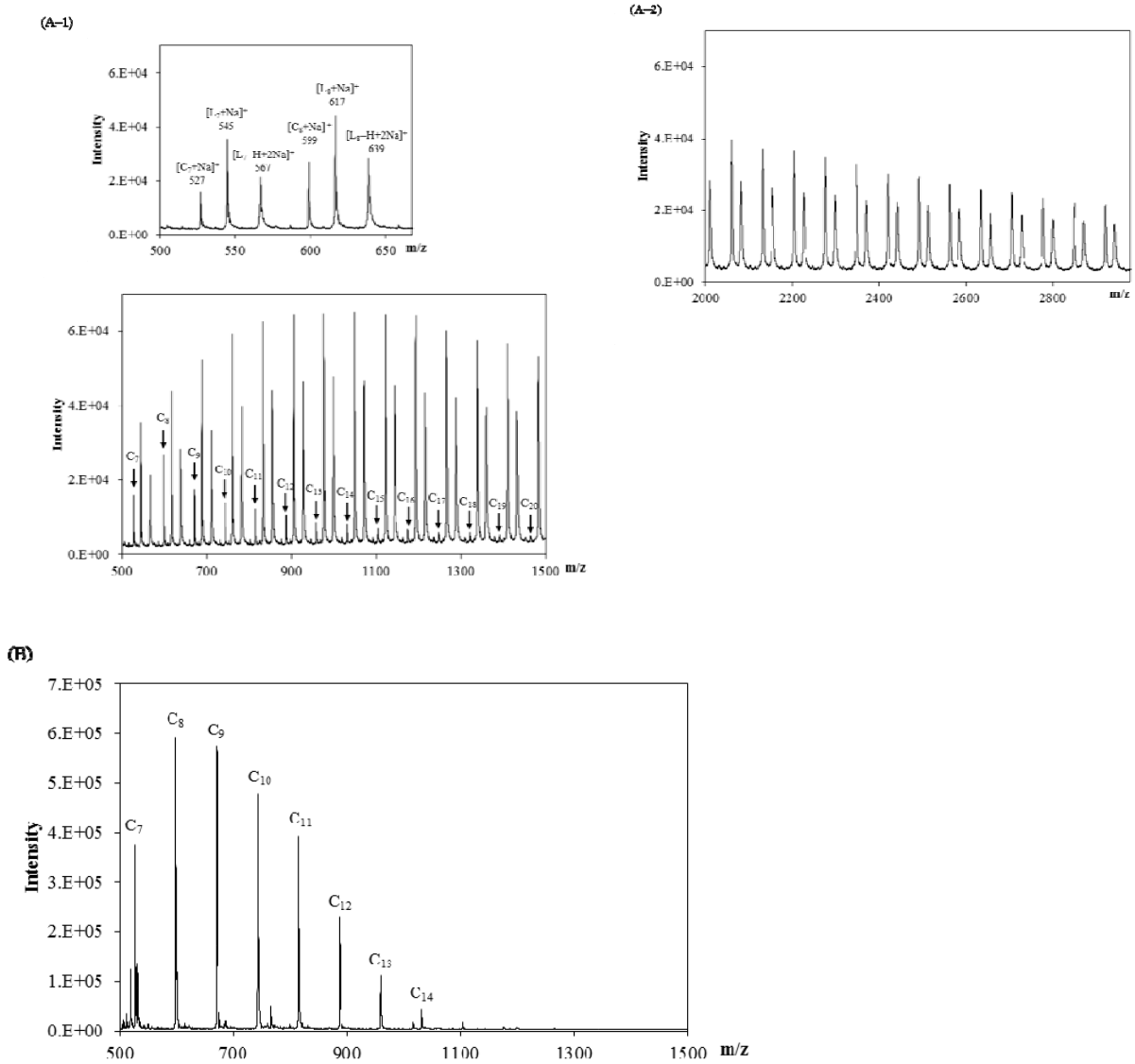

Figure 2.5 MALDI spectra of (A-1) low-molecular-weight range of PLLA (enlarged view above), (A-2) high-molecular-weight range of PLLA, and (B) the product extracted with hexane (4°C). $[L_7+Na]^+$ is due to the Na^+ adduct of l-OLLA with n = 7, $[C_7+K]^+$ is due to the K^+ adduct of c-OLLA with n = 7, and $[L_7–H+2Na]^+$ is due to the 2 Na^+ adduct without proton H^+ of l-OLLA with n = 7.

Chapter 2

Conclusion

It was confirmed that cyclic products are formed via the direct polycondensation of L-lactic acid to produce PLLA. In addition, the selective extraction of c-OLLA was successfully achieved using cold hexane or cyclohexane as the extraction solvents. The selective solubility in hexane and cyclohexane at 4°C was confirmed by ESI-MS and ^{1}H NMR analyses. The results indicated that differences in the hydrophobicity (polar character), topology, and temperature dependence of the solubility of the obtained PLLA enabled the selective extraction of c-OLLA.

The evaluation of the ESI-MS indicated that there was appreciable interaction between c-OLLA and alkali metal ions in the acetonitrile solution because c-OLLA has no end group for binding to alkali metal ions. And it is assumed that the cavity of c-OLLA attracted and included the alkali metal ions. The c-OLLA may be a novel host molecule with biocompatibility and biodegradability which can be used in human body. And it is assumed that the c-OLLA affected the direct polycondensation and disturbed molecular elongation of PLLA.

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Chapter 3

Interactions between Cyclic Oligo(L-Lactic Acid) and Alkali Metal Ions in Organic Solvent

3.1 Introduction

Poly(L-lactic acid) (PLLA) has been known for several decades as a biodegradable material. Various high molecular weight (M_w>100,000) commercial- grade PLLAs are currently used in industrial applications, including injection molding, films, and fibers. Poly(lactic acid) (PLA) is also used in biocompatible materials such as bone scaffolds and suture threads. These consist mainly of linear molecules obtained by ring opening or direct condensation polymerization. Many studies related to linear PLA [1–5] are available in the literature, while there are fewer studies on cyclic poly or oligo(lactic acid). For example, c-PLLA with molecular weight ranging from approximately 4,000–39,000 (60–540 repeat units) was synthesized using an alumatrane-inspired catalyst [6]. Shin *et al*. prepared c-PLLA by zwitterionic polymerization of lactide using N-heterocyclic carbene 1,3-dimesitylimidazol-2-ylidene as the catalyst in order to investigate the crystallinity of the polymer [7]. Therefore, this chapter focused on cyclic oligo(lactic acid) (c-OLA), as the properties of cyclic compounds are often different from those of their linear counterparts. Cyclic peptides such as depsipeptide, for example, which consist of at least one lactone unit, exhibit high stability in digestive fluids, and are thus able to remain intact even in enteron, unlike linear peptides. Cyclic aliphatic esters are generally known as macrolides, large cyclic lactones comprising more than 12 atoms. Macrolides inhibit the production of protein in bacteria, whereas linear lactone cannot [8–10]. Furthermore, cyclodextrin has been reported to induce apoptosis in cells [11], allowing c-OLA to function as an antitumor agent in humans [12, 13]. Many inclusion materials are cyclic compounds, including crown ethers and

cyclodextrins, whose inclusion properties and aforementioned effects in living organisms have potential relevance.

Some cyclic compounds are known to act as host molecules to form inclusion complexes with guest molecules. For example, crown ethers can accept various alkali metals [14]. Cyclodextrins also form inclusion complexes with a variety of guest molecules, including PLA [15–17].

As Kéki *et al*. have reported previously, c-OLA is assumed to be present in PLA [18]. In an earlier work, the author reported the extraction of cyclic oligo(L-lactic acid)(c-OLLA) using specific organic solvents in Chapter 2, in which the author succeeded in extracting only cyclic compounds from a mixture of linear and cyclic compounds, using specific organic solvents. In addition, c-OLLA was assumed to interact with guest molecules and form inclusion complexes.

In this chapter, the interaction between alkali metal ions and a mixture of various repeat units of c-OLLA extracted from PLLA was investigated. Chisholm *et al*. performed a detailed investigation of the interaction between c-OLLA with six repeat units and sodium ions [19]. An interaction was found only in the case of c-OLLA with six repeat units and sodium ions, as distinct from many other anions. However, to my knowledge, there has been no report investigating the relationship of c-OLLA with metal ions other than sodium. Thus, the interaction between each alkali metal ion and a more practical mixture of c-OLLA with various repeat units extracted from PLLA was investigated.

Chapter 3

3.2 Experiment

3.2.1 Materials

L-lactic acid (90 wt% aq. solution, HiPure 90, Purac Biochem. bv., NLD) was used as received. NaCl, KCl, RbCl, and CsCl (Nacalai Tesque, Inc., JPN) were used as received as the source of guest ions. Polycondensation of L-lactic acid PLLA was synthesized using the direct condensation polymerization method, as reported previously [20], with the exception that no catalyst was added. A 300-ml three-neck separable flask was equipped with a magnetic stirrer and reflux condenser connected to a vacuum system through a cold trap. First, the flask was charged with L-lactic acid (100 g) and heated in stages to 160°C while stirring. Simultaneously, the pressure was reduced stepwise to 4 kPa, at which point the reaction was continued for 21 h. As the reaction proceeded, the solution gradually became viscous. When the reaction was complete, the flask was cooled to room temperature (26°C), and the product was crushed to a powder using a mortar.

3.2.2 Isolation of cyclic oligo(L-lactic acid)

Powdered PLLA (5 g) was added to toluene (50 ml; Nacalai Tesque Inc.), and insoluble materials were removed from the mixture by filtration with filter paper (0.5 μm), and the filtrate then washed sequentially with saturated aqueous $NaHCO_3$, distilled water, and saturated aqueous NaCl. The toluene phase was separated, filtered, and evaporated to afford a powder (50–70 mg). The extraction yield of cyclic products was approximately 1.3 wt%.

These products were then analyzed by electrospray ionization mass spectrometry (ESI-MS), proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), following solvent removal under a vacuum at 40°C

3.2.3 Liquid–liquid ion transformation

The complexation efficiency of c-OLLA was evaluated by measuring the distribution of colored sodium picrate between an immiscible mixture of organic solvent and water. To determine a standard line, three concentrations of aqueous sodium picrate solution (1, 0.5, and 0.25 mM) were prepared and their absorbance measured at λ = 354 nm. In a conical flask (50 ml), chloroform (10 ml; Kanto Chemical Co., Inc., JPN) containing c-OLLA (50 mg, estimated as 5.0 mM) and aqueous sodium picrate (10 ml, 1.0 mM) were vigorously stirred with a magnetic stirrer for 12 h. PLLA (50 mg, synthesized in 2.2) was used in place of c-OLLA for comparison. A blank measurement was also conducted without c-OLLA or linear oligo(L-lactic acid) (l-OLLA). The concentration of sodium picrate was determined using a spectrophotometer (ASV11D; AS ONE Co., JPN) at λ = 354 nm.

3.2.4 Determination of optical purity

The optical purity of the lactic acid units was determined by high-performance liquid chromatography (HPLC, LC-20 Series; Shimadzu Co., JPN) equipped with an optical resolution column (MCI GEL CRS10W, Mitsubishi Chemical Co., JPN). Prior to analysis, a polymer sample (1.5 mg) was dipped in NaOH (0.5 ml, 2 M) for hydrolysis at room temperature. The hydrolysate was then neutralized with H_2SO_4 (0.5 ml, 1 M), diluted by 10 %, and injected into the analyzer. The analytical conditions were as follows: column temperature, 35°C; elution solvent, 1 mM aqueous $CuSO_4$ solution; flow rate, 0.5 ml/min. Both L- and D-lactic acids were detected with a UV detector at a wavelength of 254 nm. Optical purity was evaluated by calculating the enantiomeric excess (ee) percentage:

$$\%ee = 100 \times ([L] - [D])/([L] + [D])$$

where [L] and [D] denote the molarities of L- and D-lactic acids, respectively.

3.2.5 Mass spectrometry of extracted products

ESI-MS spectra were recorded using an ion trap mass spectrometer (amaZon SL; Bruker Daltonics Inc., USA). The operating conditions were as follows: flow rate, 240 μl/h; capillary temperature, 175°C; spray voltage, 4.5 kV; capillary voltage, 40 V. An acetonitrile (LiChrosolv; Merck Millipore, DEU) solution of c-OLLA (10 ppm, 200 μl) was mixed with an aqueous solution of NaCl, KCl, RbCl, and CsCl (100 ppm, 200 μl each) and filtered with a 0.45 μm filter.

3.2.6 Tandem mass spectrometry of extracted products

ESI-MS/MS spectra were recorded using an ion trap mass spectrometer (amaZon SL; Bruker Daltonics Inc.). The precursor ion was generated by ESI-MS. An acetonitrile solution of c-OLLA (10 ppm, 200 μl) was filtered with a 0.45 μm filter prior to injection. The other operating conditions were the same as those listed for the ESI-MS method. For MS/MS experiments, helium was used as the collision gas, with a collision voltage (fragment amplifying) of 0.2 to 3.0 V.

3.2.7 NMR analysis

^{1}H NMR spectra were recorded using an AVANCE Series 300 MHz spectrometer (Bruker BioSpin Co., USA). Samples were dissolved in acetonitrile-d3 and D_2O, and the spectra were recorded at 23°C. An acetonitrile-d_3 solution (750 μl) containing c-OLLA (5 mg) and a D_2O solution (100 μl) containing alkali metal chloride (2 mg, NaCl, KCl, RbCl, or CsCl) were mixed and analyzed.

3.2.8 Mass spectrometry of polymers and extracted products

MALDI spectra were recorded using an autoflex speed-KF system (Bruker Daltonics Inc.) equipped with a nitrogen laser (Smartbeam-II, 355 nm). The acceleration voltage was 19

kV, and the spectra were obtained in linear, positive ion mode with pulsed ion extraction (120 ns). The energy of the laser beam was set at approximately 70%, and trans-3-Indoleacrylic acid was used as the matrix and sodium iodide as the cationization agent for the PLLA and extracted c-OLLA. External calibration was performed with appropriate samples of angiotensin II (MALDI-MS calibrant; Wako Pure Chemical Ind., JPN) using α-cyano-4-hydroxycinnamic acid (Wako Pure Chemical Ind.) as the matrix and trifluoroacetic acid (Nacalai Tesque, Inc.) as the cationization agent. The samples were measured employing a solvent-free MALDI-MS method.

3.3 Results and Discussion

3.3.1 Extraction of cyclic oligo(L-lactic acid)

PLLA was synthesized via direct polycondensation. The average number of repeat units of PLLA was found to be approximately 16, which was estimated by the ratio of endgroup methine protons (δ = 4.2 ppm) to the main chain methine protons (δ = 5.0, 5.1, 5.2 ppm) derived from the ^{1}H NMR spectrum. The optical purity of the PLLA was 96.0%ee, while that of raw L-lactic acid was 99.9%ee. PLLA was dissolved in toluene, and the linear oligomers with end groups were removed as sodium salts by the acid-base reaction between carboxyl end groups and $NaHCO_3$. This was performed according to the method reported by Zhang *et al.* for obtaining 3-hydroxypropanoic acid macrocyclic esters [21]. The yield of the extracted products was 1.3%, as determined using gravimetric methods. The extracted products were evaluated using ^{1}H NMR, which showed no signals derived from end groups (δ = 5.0 ppm, data not shown). Therefore, the extracted products were assumed to be cyclic. In addition, MALDI-MS was performed to confirm the number of repeat units in each molecule. MALDI-MS has been used as an effective method for mass analysis of synthetic polymers because it is soft enough to provide molecular ions without fragmentation, thus affording the

mass of intact polymer ions by cationization, providing access to an essentially unlimited mass range [22]. The spectra of extracted products showed no peaks derived from linear oligomers, but displayed those for cyclic oligomers, while PLLA before extraction showed peaks for both linear and cyclic oligomers (Figure 3.1). The results of ^{1}H NMR spectroscopy and MALDI-MS indicated that the extracted products were c-OLLA with molecular weights below 1,500 (21 or fewer repeat units).

3.3.2 Interaction in solution

As reported previously, c-OLLA has the capability of forming inclusion complexes in solvent (Chapter 2). To analyze the behavior of c-OLLA in solution, ESI-MS was conducted in the presence of various ions including sodium, potassium, rubidium, and cesium with c-OLLA in acetonitrile and aqueous solutions through the addition of chloride salts of alkali metals (Figure 3.2). Each ion adduct peak is emphasized in Figure 3.3. Table 3.1 shows the relationship between the number of repeat units and alkali ion adducts. It can be seen that larger ions tend to form adducts with larger c-OLLA.

Table 3.1 The relation between adducted alkali ions and the number of repeat units

Adducted alkali ions	Diameter (Å)	c-OLLA the number of repeat units[a)]
Na^+	1.94	6, 7
K^+	2.66	8, 7
Rb^+	2.94	8, 9
Cs^+	3.34	9, 10

a) 1st and 2nd largest signals

Chapter 3

In ESI-MS, raw ions that exist in solution are vaporized to form small charged droplets by Coulomb repulsion, and the ESI spectrum therefore reflects the interaction between the ions in the solution and the sample. In other words, the spectrum intensity reflects the intensity of the interaction between ions and sample in solution. With this taken into account, the results of ESI-MS indicated that c-OLLA with n = 6 or 7 and n = 9 or 10 repeat units interacted most strongly with sodium and cesium ions, respectively. Thus it is assumed that there is a positive correlation between the ion size and inner diameter of c-OLLA for sodium, potassium, rubidium, and cesium.

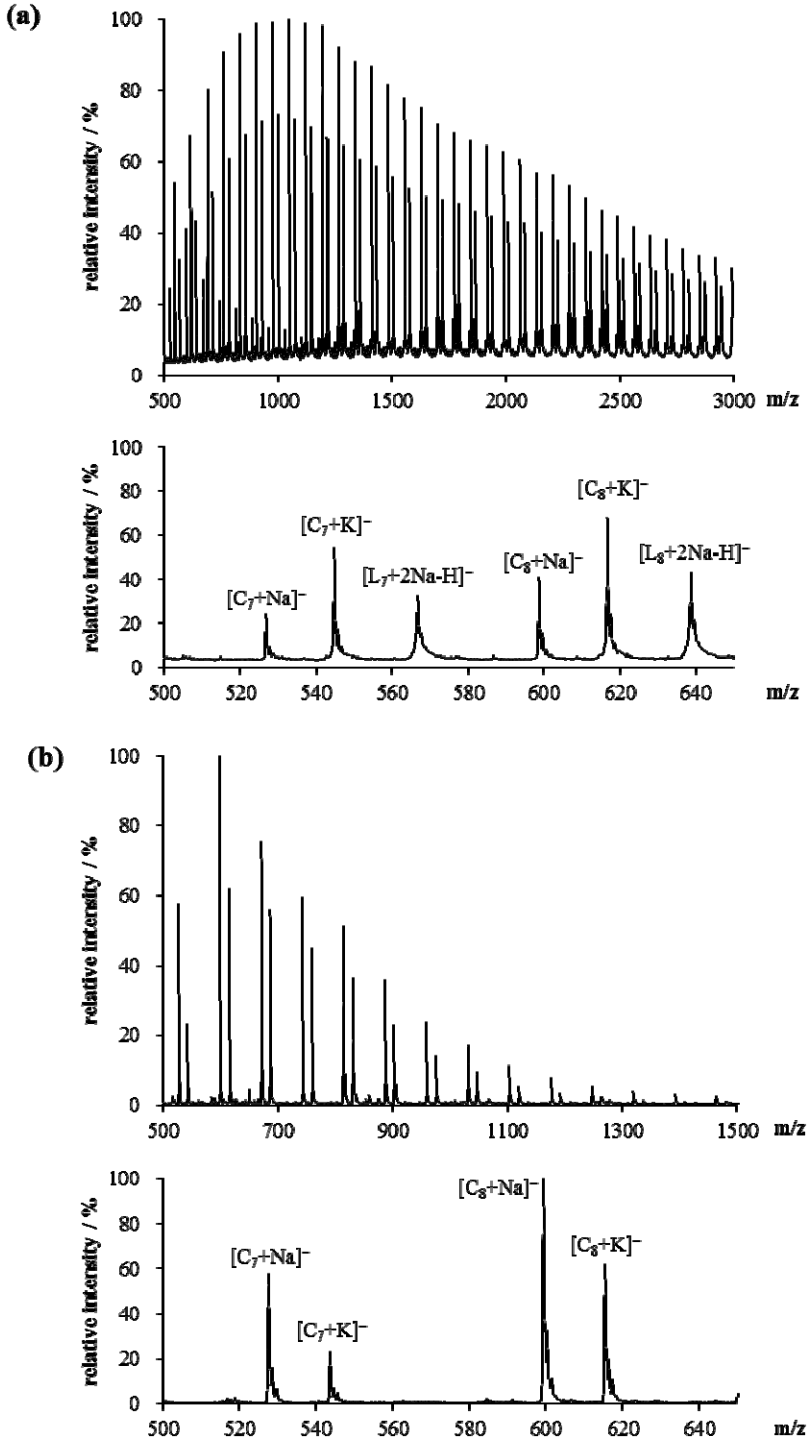

Figure 3.1 MALDI-MS spectra of (a) PLLA before extraction and (b) the extracted product. $[C_7 + Na]^+$ is the sodium ion adduct of c-OLLA with $n = 7$, and $[L_7 + 2Na–H]^+$ is the $2Na^+$ adduct and proton loss of c-OLLA with $n = 7$.

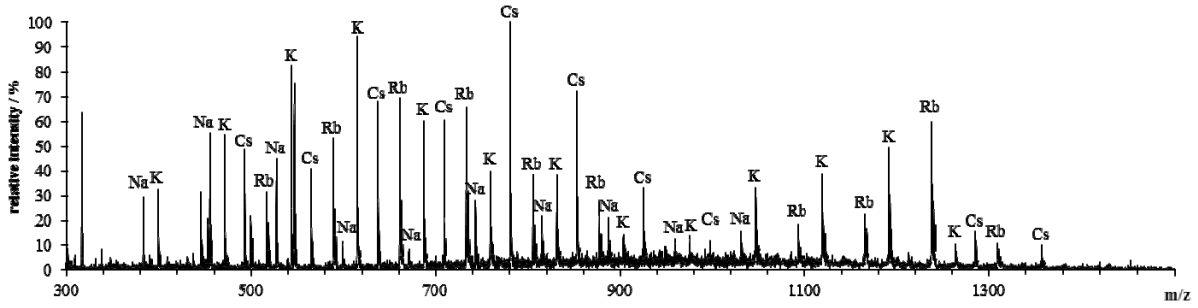

Figure 3.2 ESI-MS spectrum of c-OLLA with four different alkali metal ions.

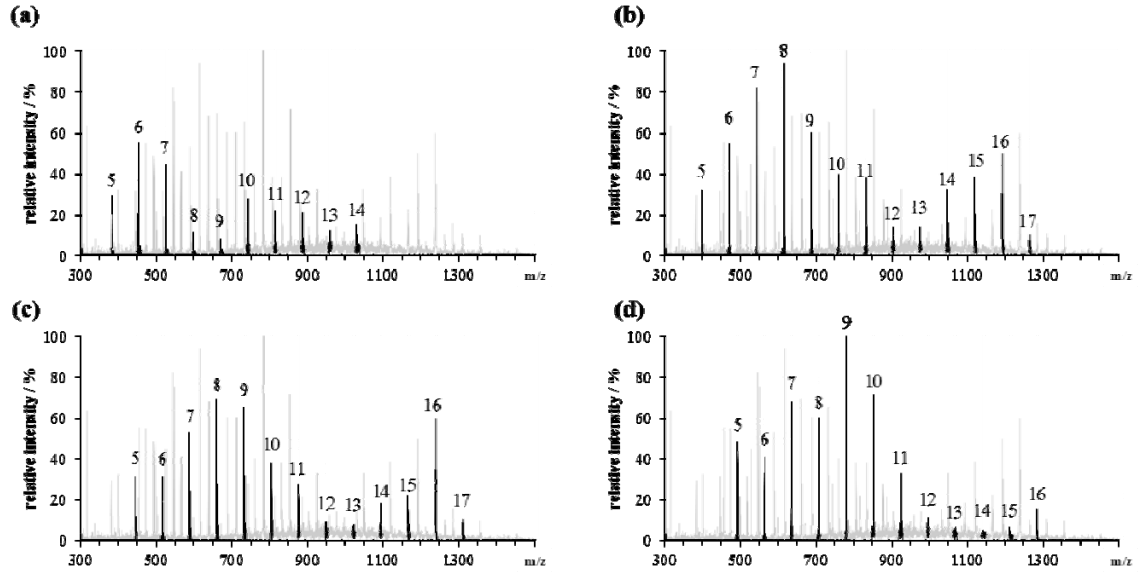

Figure 3.3 ESI-MS spectra of c-OLLAs with (a) sodium ion adduct, (b) potassium ion adduct, (c) rubidium ion adduct, and (d) cesium ion adduct. These specific signals were emphasized by diluting other signals. The numbers indicate the number of repeat units.

In addition, MALDI-MS was conducted by adding each alkali ion, i.e., NaCl, KCl, RbCl, and CsCl, as the ionizing agent in place of NaI (Figure 3.4). Each ion adduct was detected without any change in molecular weight composition. The results of MALDI-MS reflected interaction in a bulk state, while the results of ESI-MS reflected interaction in specific solution. Therefore, a correlation between ion size and the inner diameter of c-OLLA exists only in solution, and not in bulk.

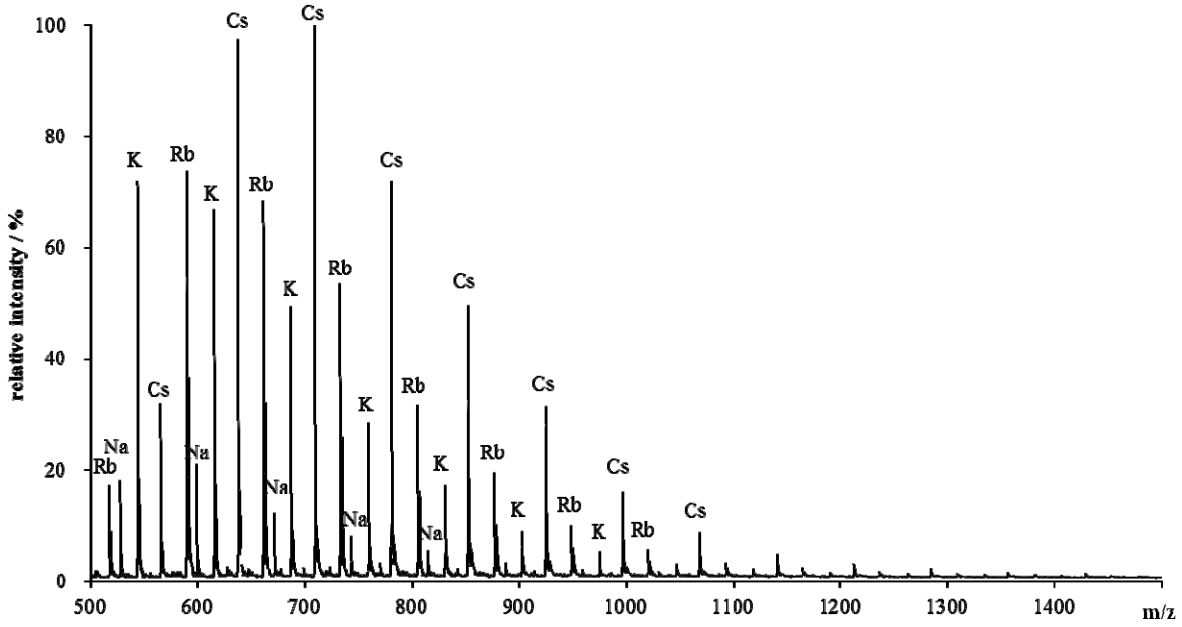

Figure 3.4 MALDI-MS spectrum of c-OLLA with four different alkali metal ions.

Some cyclic compounds form inclusion complexes such as crown ethers or cyclodextrins. Various polyamine and polyketone cyclic compounds are also known to form inclusion complexes [23–26], as their carbonyl groups attract guest ions. It is assumed that carbonyl groups in c-OLLA attract alkali metal ions in solution as they do in polyamines and polyketones; however, c-OLLA differs from polyamines and polyketones in that it has good biocompatibility, biodegradability, and bioavailability.

The relationship between the inner diameter of the host cyclic molecule and the diameter of the guest molecule is an important factor in the ability of cyclic compounds to form inclusion complexes. In this chapter, c-OLLA included alkali ions in its inner cavity is assumed. Linear molecules are known to be more flexible and can change shape more easily than their cyclic counterparts. Cyclic molecules are able to form adducts with specific ions, such as sodium, by simply shifting their carbonyl groups to the inside of the cavity. In contrast, linear molecules must completely change in shape to form an adduct with a specific ion,

which is highly unfavorable with regard to entropy (Figure 3.5). In solution, l-OLLA is in a higher entropy state than c-OLLA. Once they form ion adducts, their entropies decrease to become equivalent. Thus, the entropy gap of l-OLLA before and after adduct formation is significantly larger than that of c-OLLA, implying that adduct formation with l-OLLA involves a considerably larger loss of entropy, which is unfavorable. Ion adducts are assumed to be included in the inner cavity of the cyclic compounds during ionization.

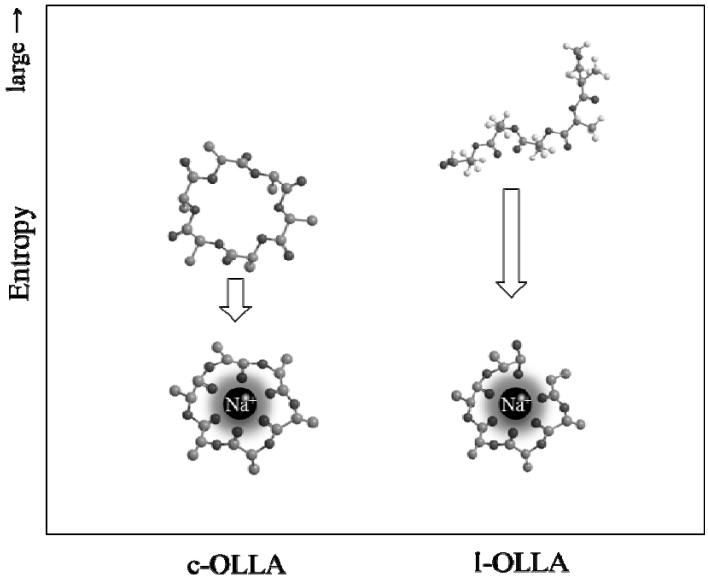

Figure 3.5 The entropy of c-OLLA and l-OLLA before and after conformation change in solution.

3.3.3 Molecular weight of cyclic oligo(L-lactic acid) with sodium ion

Furthermore, focusing on the distribution of c-OLLA molecular weight in the ESI-MS spectrum (Figure 3.6), the distribution was completely unlike that of MALDI-MS (Figure 3.1(b)). The ESI-MS spectrum showed bimodal distribution, with intense peaks observed in the higher m/z area, suggesting that the c-OLLA configuration in solution was different from that in bulk, as measured by MALDI-MS. To clarify this point, ESI-MS/MS was conducted. MS/MS spectra of c-OLLA were measured with a collision voltage of either

0.4 V or 3.0 V, as shown in Figures 3.7 and 3.8, respectively. The intense signals observed under a collision voltage of 0.4–3.0 V are also shown in Table 3.2. The collision voltage threshold for fragmentation was 0.2–0.4 V, as no fragmentation occurred below 0.2 V. Figures 3.7(a), (b), (c) and (d) show MS/MS spectra of $[M_6+Na]^+$, $[M_{12}+Na]^+$, $[M_{13}+Na]^+$, and $[M_{14}+Na]^+$, respectively, at 0.4 V collision voltage, where M_n indicates molecules with "n" repeat units. At this collision voltage, a certain amount of precursor ions still remain intact. Notably, the main product was detected as the sodium ion adduct of n = 6 for $[M_{12}+Na]^+$. Similarly, sodium ion adducts of n = 7 were detected for $[M_{13}+Na]^+$ and $[M_{14}+Na]^+$, while n = 5 and 4 were detected for $[M_6+Na]^+$. These results suggest that some signals (over n = 10) detected by ESI-MS were not the exact molecular weight when MALDI-MS analysis was taken into consideration. Specifically, each c-OLLA above n = 11 was assumed to form a complex with sodium ions in solution. The complex is held together by non-covalent bonds; therefore, the low collision voltage caused splitting into single c-OLLA without cleaving covalent bonds. For instance, the precursor ion of n = 12 may consist of two molecules of c-OLLA (n = 6), which can be shown as $[2\ M_6+Na]^+$. In addition, c-OLLA below n = 10 does not possess a complex structure, as only the sodium ion adducts, which were formed by the loss of one or two monomeric lactic acid units from c-OLLA, were observed as MS/MS product ions. Figure 3.8 shows the ESI-MS/MS spectrum of c-OLLA precursor ions of n = 12 at 3.0 V collision voltage. Almost all precursor ions were found to be fragmented, while no fragment peaks were detected in the ESI-MS/MS spectra of c-OLLA at 0.2 V collision voltage. These results suggest that the collision voltage threshold for fragmentation of $[2\ M_n+Na]^+$ was between 0.2 and 0.4 V. Osaka *et al*. also reported that the intense peaks in the MS/MS spectrum of PLA (n = 12) were $[M_{11}+Na]^+$ and $[M_{10}+Na]^+$ [27].

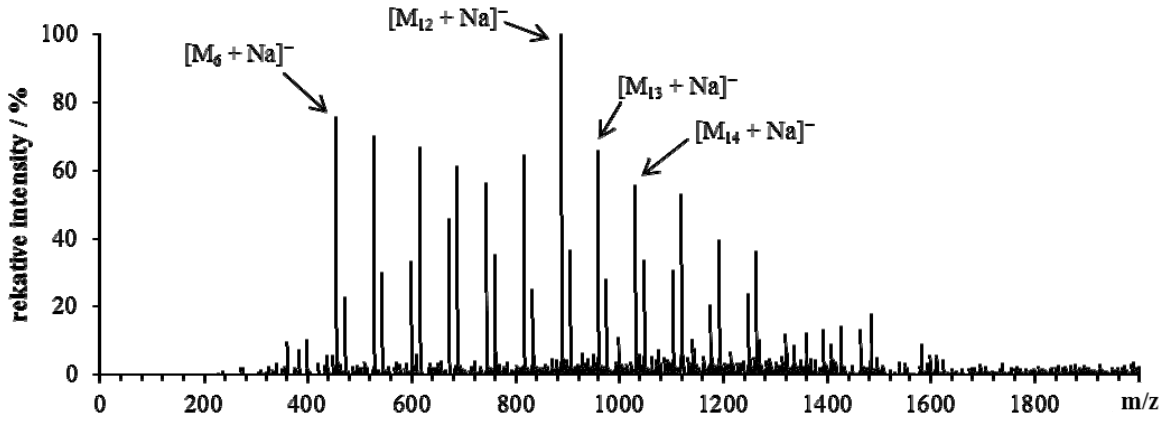

Figure 3.6 ESI-MS spectrum of c-OLLA.

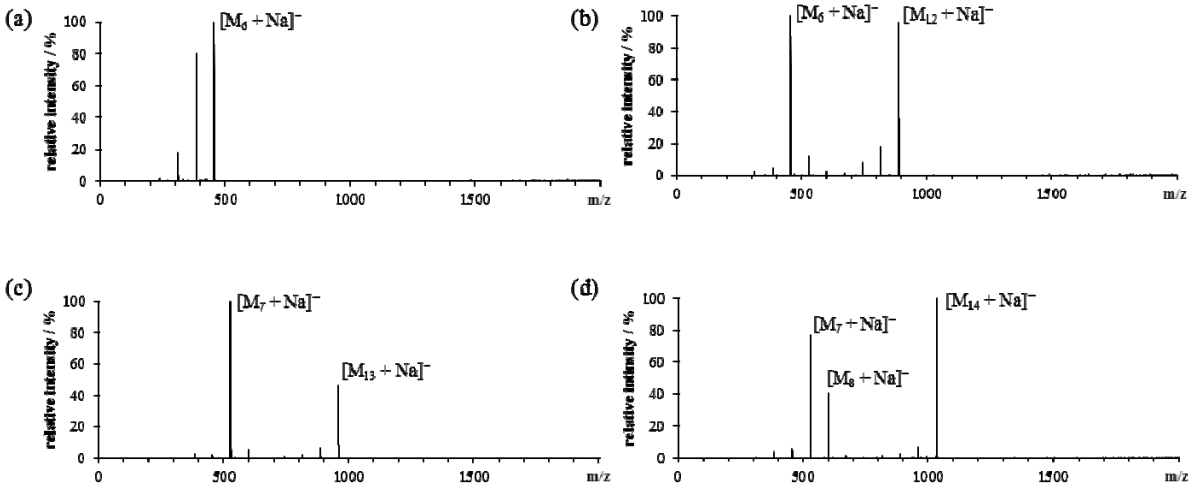

Figure 3.7 ESI-MS/MS spectra of c-OLLA precursor ions of (a) n = 6, (b) n = 12, (c) n = 13, and (d) n = 14 at 0.4 V collision voltage.

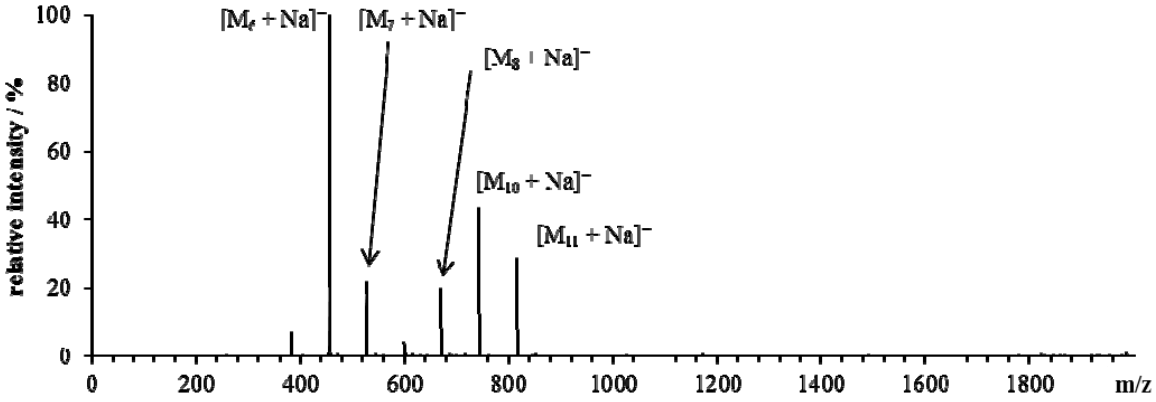

Figure 3.8 ESI-MS/MS spectrum of c-OLLA precursor ions of n = 12 at 3 V collision voltage.

Table 3.2 Collision voltage and number of repeat units of fragmented ions in ESI-MS/MS

Collision voltage (V)	Number of repeat units of precursor ion										
	16	15	14	13	12	11	10	9	8	7	6
0.2	16	15	14	13	12	11	10	9	8	7	6
0.4	16	8,15	7,14	7,13	6,12	11,6	10	9,8	8	7,6	6,5
0.6	16	8,9,6	7,8,13, 12	7,13	6,11	10,6,9	9,8, 10	9,8	8,7	6,5	5,4
0.8	8,9, 16	8,9	7,8,13, 12	7	6,11	10,9,6	9,8	8	7	6,5	5,4
1.0	8,9	8,9	7,8,13, 12	7	6,11	10,9,6	9,8	8	7,6	6,5	5,4
3.0	8,9,7	9,8, 13,12	7,8,12	7	6,10	9,6,10	8,9	8,7	7,6	6,5	5,4

3.3.4 Liquid–liquid ion transformation

As ESI-MS and MS/MS indicated the affinity of various alkali metal ions for c-OLLA, a liquid–liquid ion transformation experiment was conducted for direct observational verification. Sodium picrate was used as the alkali ion source. The change in the amount of picrate ion in the aqueous phase after mixing is shown in Figure 3.9. The amount of picrate ion reflects the amount of sodium ion. The distribution coefficient was estimated by the following calculation: $(C_I - C_F)/C_I$, where C_I and C_F denote the initial and final sodium picrate concentrations, respectively. The distribution coefficient calculated from the results of Figure 3.9 at 0 and 11.5 h were 11.4 and 6.9% when c-OLLA and PLLA were added, respectively, and 4.1% with no addition. This explains the adduct formation of c-OLLA with sodium ions and indicates that c-OLLA caused the transport of more sodium ions to the organic phase than PLLA.

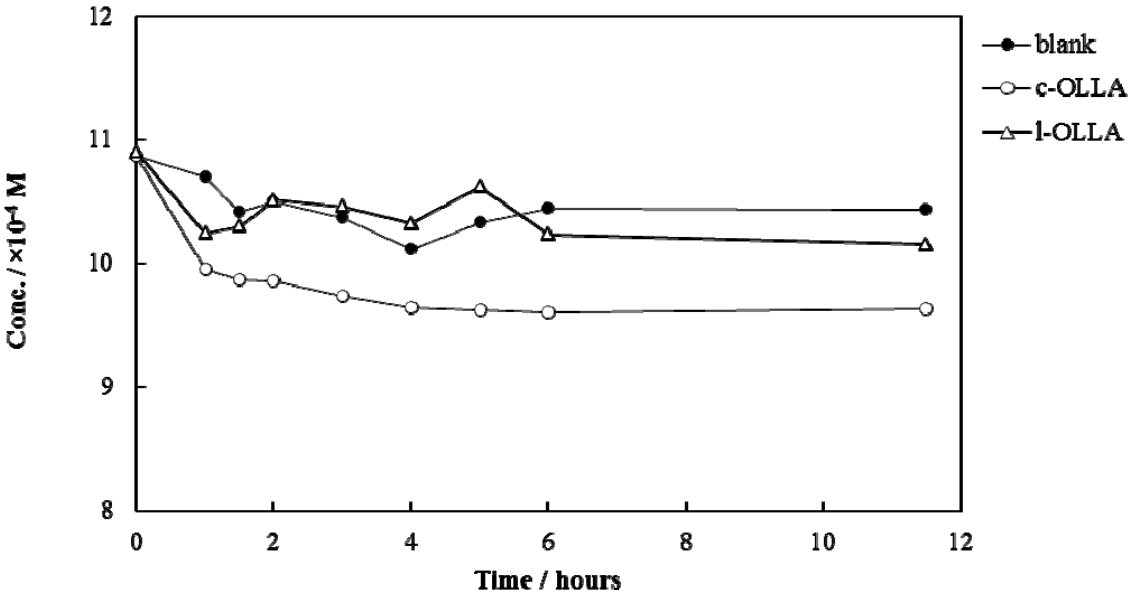

Figure 3.9 Change in picrate concentration in the aqueous phase.

Chapter 3

Conclusion

In this work, the extraction of c-OLLA was achieved by removing linear oligomers from PLLA. ESI-MS was used to investigate the interaction between c-OLLA and alkali metal ions, including sodium, potassium, rubidium, and cesium. The analysis was conducted in the presence of a mixture of c-OLLA with various repeat units and four different alkali metal ions. A positive correlation was found between ion size and the inner diameter of c-OLLA, as well as a positive interaction between c-OLLA and alkali metal ions. Furthermore, ESI-MS/MS analysis suggested that two kinds of c-OLLA formed adducts with sodium ions in solution. Liquid–liquid transformation of sodium ions using the interaction with c-OLLA was also achieved, for the first time. From this, it was found that c-OLLA expressed higher complexation efficiency than PLLA by extracting sodium ions from the aqueous phase to the organic phase. c-OLLA differs from other cyclic compounds, such as crown ethers, polyamines, and polyketones, as it has good biocompatibility and biodegradability. Thus, it appears that the transport of ions to specific sites using c-OLLA in living organisms is not only possible, but a safer alternative to conventional drug delivery systems.

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Chapter 4

General Conclusion

The purpose of this study complied in this thesis were research of synthesis and properties of cyclic oligo(lactic acid).

The essence of each chapter is as follows.

Chapter 1 described the background of this thesis, example of synthesis and extraction of cyclic polyester and oligoesters. Properties and functions of polyester and oligoester concentrating on cyclic oligo(lactic acid) are also introduced.

In Chapter 2, the extraction of cyclic oligo(lactic acid) from direct polycondensation PLLA with organic solvent was reported. The contents of poly(L-lactic acid) (PLLA) prepared by direct condensation polymerization without using a catalyst were studied. ^{1}H NMR and mass spectrometry analyses suggested that PLLA contained cyclic oligo(L-lactic acid) (c-OLLA) with 3–20 repeat units. Notably, only c-OLLA was extracted and isolated using hexane or cyclohexane at 4°C; thus the hydrophobicity, topology, and temperature dependence of the solubility of the obtained PLLA enabled the selective extraction of c-OLLA. The effect of cyclic compounds on direct polycondensation and the potential for c-OLLA to form molecular inclusion complexes were also discussed.

In Chapter 3, the interactions between cyclic oligo(L-lactic acid) and alkali metal ions in organic solvent were reported. The interaction between cyclic oligo (L-lactic acid) (c-OLLA) and alkali metal ions was investigated, including sodium, potassium, rubidium, and cesium. The analysis was conducted by electrospray ionization mass spectrometry (ESI-MS) and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) in the presence of a mixture of c-OLLA with 21 or fewer repeat units, which was extracted from poly(L-lactic acid), and alkali metal ions. The results suggested that an interaction between

c-OLLA and alkali ions exists only in solution, not in bulk. In addition, ESI-MS/MS analysis of higher m/z analytes suggested that formation of two kinds of c-OLLA with sodium ions occurred, and thus it is assumed that c-OLLA includes alkali ions in its inner cavity. The liquid–liquid ion transformation in the presence of sodium ions and c-OLLA further supports this interaction. c-OLLA was found to exhibit higher complexation efficiency than PLLA by extracting sodium ions from the aqueous phase to the organic phase.

Publication List

1. **Synthesis and Function of Cyclic Poly(Lactic Acid) and Oligo(Lactic Acid)**, Keiichiro Nomura, Hitomi Ohara, *Mini-Reviews in Organic Chemistry*, **2017**
   DOI: 10.2174/1570193X13666161102154649

2. **Cyclic Oligolactic Acid in Direct Polycondensation PLLA and Its Extraction with Organic Solvent**, Keiichiro Nomura, Yuta Nakatsuchi, Ryugo Shinmura, Sommai Pivsa-Art, Weraporn Pivsa-Art, Yuji Aso, Hitomi Ohara, *Journal of Polymers*, **2014**
   DOI: 10.1155/2014/830137

3. **Interactions between Cyclic Oligo(L-Lactic Acid) and Alkali Metal Ions in Organic Solvent**, Keiichiro Nomura, Ryugo Shinmura, Weraporn Pivsa-Art, Wichean Khawdas, Yuji Aso, Hitomi Ohara, *Journal of Polymer Research*, **2015**
   DOI: 10.1007/s10965-015-0826-z

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Keiichiro Nomura