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Synthesis of Aliphatic Poly(ester-amide)s by Ring-Opening Polymerization of L-Lactide and Amino Acid Anhydrides

Cholticha Noomhorm[a*] and Yutaka Tokiwa[b]

[a] Department of Chemistry, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.
[b] National Institute of Advanced Industrial Science and Technology (AIST), Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan.

* Corresponding author, E-mail: fscictn@ku.ac.th

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ABSTRACT: Poly(ester-amide)s were prepared by ring-opening copolymerization of L-lactide with glycine anhydride to form poly(lactide-*co*-glycine) and with alanine anhydride to form poly(lactide-*co*-alanine). At 195°C there were about 5.7 mol% inclusion of glycine using potassium tert-butoxide as catalyst in poly(lactide-*co*-glycine) and 5.0 mol% inclusion of alanine using tin(II) bis-2-ethylhexanoate as catalyst in poly(lactide-*co*-alanine). Enzymatic degradation studies showed that both copolymers could be degraded by proteinase K and glycine and alanine were included in their respective copolymers.

KEYWORDS: amino acid anhydride, biodegradable, L-lactide, proteinase K, ring-opening polymerization.

INTRODUCTION

Development of biodegradable polymers having comparable properties to replace the existing non-biodegradable polymers has been a subject of considerable interest in recent years due to the environmental problems that have emerged from waste disposal[1]. Aliphatic polyester is one well-known biodegradable synthetic polymer[2-6] and several aliphatic polyesters are now commercially available, such as poly(L-lactide). It is known as an excellent biocompatible and biodegradable polymer, however, its application is limited as it lacks physical, mechanical, thermal and processing properties[7]. Various solutions are being proposed to optimize their properties without losing any biodegradability. One of the solutions is to copolymerize the ester linkages in poly(L-lactide) with the amide linkages as poly(ester-amide) (PEA)[8-9]. Polyamides are high performance polymers due to their unique combination of thermal, physical and mechanical properties, which result from the hydrogen bonding interactions between neighboring chains of the amide linkages. Amide linkages of synthetic polymers, such as nylons are usually considered as non-biodegradable, but amide linkages from α-amino acids, which are found in protein, are easily cleaved by enzymes[10-11]. Thus, in order to modify properties of poly(L-lactide) and to control its degradation behavior, inclusion of α-amino acids in the biodegradable poly(L-lactide) main chain is required.

Several poly(ester-amide)s (PEAs) containing α-amino acids have been synthesized and the effects of the α-amino acid building blocks in PEAs main chain on biodegradability was reported[12-14]. PEAs based on 1,2-ethanediol, adipic acid and amino acids such as glycine, leucine, phenylalanine were synthesized and the degradation studies indicated that polymers containing glycine were not degraded with proteolytic enzymes (chymotrypsin and elastase)[15]. Series of PEAs derived from glycine[16] and alanine[17] derivatives were synthesized by interfacial polymerization. Studies on the biodegradation and biocompatibility of the synthesized polymers showed that only the alanine-dodecanediol-alanine-sebacic acid sequence is susceptible to enzymatic degradation, which can also be controlled by varying the L- and D-alanine ratio[18]. Extended degradation studies of these PEAs based on alanine-dodecanediol-alanine-1,12-dodecanedioic acid showed that papain and proteinase K are the most effective proteolytic enzymes and the hydrolytic degradation takes place through the ester linkages[19]. PEAs based on bis(α-amino acid), α,ω-alkylene diesters and aliphatic dicarboxylic acids concluded that the higher the overall hydrophobicity of the polymer backbone, the more sensitive it was toward the α-chymotrypsin-catalyzed hydrolysis[20]. Ring-opening copolymerization of cyclodepsipeptides (morpholine-2,5-dione and its derivatives) with D-lactide, L-lactide, glycolide or ε-caprolactone to prepare PEAs was reported[21]. Alternative and random PEAs are obtained by homopolymerization of morpholine-2,5-dione and copolymerization of morpholine-2,5-dione derivatives with caprolactone or lactides, respectively. In the case of copolymerization with L-lactide, the melting

temperature and crystallinity rapidly decreased with increasing morpholine-2,5-dione content and the degradation rate varied with the morpholine-2,5-dione content.

An attempt was made in this study to find an alternative route to prepare poly(ester-amide)s by following the idea of the successive ring-opening polymerization of the six-membered cyclic dimer glycolides and lactides to prepare poly(glycolic acid) and poly(lactic acid) together with the ring-opening copolymerization of cyclodepsipeptides to prepare the PEAs. Thus, ring-opening polymerization of cyclic anhydrides from amino acids was investigated to see the potential as an alternative means of preparing the poly(ester-amide)s, and then the susceptibility of the products to enzymatic hydrolysis by proteinase K was studied.

Materials and Methods

Materials

L-Lactide from PURAC Biochem BV (The Netherland), glycine anhydride or piperazine-2,5-dione from Tokyo Chemical Industry (Japan), alanine anhydride or 3,6-dimethyl piperazine-2,5-dione (99% mixture of DL and *meso*) and 1.0 M potassium tert-butoxide (t-BuOK) in THF from Aldrich (U.S.A.), proteinase K from *Tritirachium album* from ICN Biomedicals, Inc.(Germany), dibutyltin(IV) diacetate [$(Bu)_2Sn(CH_3COO)_2$] from Wako Pure Chemical Industries (Japan), and tin(II) bis-2-(ethylhexanoate) ($Sn(OCt)_2$) from Nacalai Tesque (Japan) were used without any further purification.

Methods

^{1}H NMR spectrum was recorded on JEOL JNM-EX270 (270.05MHz) spectrometer using tetramethylsilane (TMS) as the internal reference. The thermal analysis was obtained on a differential scanning calorimeter (DSC) 220C (Seiko Scientific Instruments) with a heating rate of 10°C/min. The apparent molecular weight of copolymers was estimated in $CHCl_3$ by gel permeation chromatography (GPC) with TSK-Gel Multipore H_{XL}-M column and refractive index detector (Tosoh Co., Japan). Analysis of the water-soluble total organic carbon (TOC) concentration after filtration with a 0.20 mm filter membrane (Millipore Co., U.S.A.) was measured with a TOC-5000A analyzer (Shimadsu Co., Japan).

Ring-opening copolymerization of L-Lactide and Glycine Anhydride or Alanine Anhydride

L-Lactide (0.58 g, 4 mmol; 0.86 g, 6 mmol and 1.30 g, 9 mmol), glycine anhydride (0.11 g, 1 mmol; 0.46 g, 4 mmol and 0.68 g, 6 mmol) or alanine anhydride (0.14 g, 1 mmol; 0.57 g, 4 mmol and 0.85 g, 6 mmol) were copolymerized with the mole ratios of L-lactide: glycine anhydride or alanine anhydride of 9:1 mmol, 6:4 mmol and 4:6 mmol. Catalysts such as $Sn(OCt)_2$ (0.004 g, 0.01 mmol for comonomer:catalyst =1000:1), $(Bu)_2Sn(CH_3COO)_2$ (0.0035 g, 0.01 mmol for comonomer:catalyst =1000:1) and t-BuOK (0.2 mL of 1.0 M, 0.2 mmol for comonomer:catalyst =50:1) were added at varied mole ratios of comonomer:catalyst, including 50:1 mmol, 150:1 mmol, 500:1 mmol and 1000:1 mmol. The reaction components were transferred into an ampule, which was sealed under vacuum after purging 3 times with nitrogen at room temperature (when t-BuOK was used as catalyst, the desired amount of 1.0 M t-BuOK in THF was transferred into an ampule and the ampule was sealed under vacuum after THF was evacuated and the ampule purged 3 times with nitrogen at room temperature). The ampule was put in the oven at 130°C for 5 days or 170°C for 2 days or 195°C for 1 h and then opened.

After the ampule was opened, the mixture was dissolved with $CHCl_3$ and the unreacted anhydride which was insoluble in $CHCl_3$ was filtered off . The $CHCl_3$ portion was concentrated with a rotary evaporator, precipitated in hot water, dried under vacuum for 24 h and slightly yellow puffy masses of poly(lactide-*co*-glycine) and poly(lactide-*co*-alanine) were obtained.

^{1}H NMR ($CDCl_3$) of homopoly(L-lactide), PLLA (mole ratio of monomer:$Sn(OCt)_2$ = 1000:1 at 195°C for 1 h) δ = 1.6 (d, 3H, -CH-C<u>H</u>$_3$), and 5.2 (q, 1H, –C<u>H</u>-CH$_3$) (Fig. 1a); DSC: melting point at 168.6°C; GPC: $\overline{M}_n$ =16300, $\overline{M}_w/\overline{M}_n$ = 1.86.

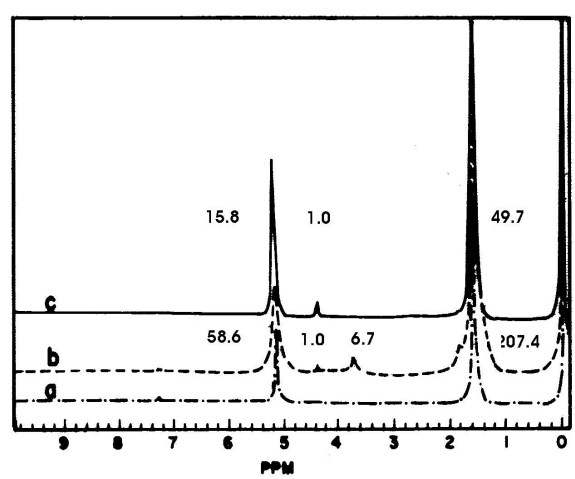


Fig 1. ^{1}H NMR ($CDCl_3$) spectra of (a) poly(L-lactide) (b) poly(lactide-*co*-glycine) using t-BuOK as catalyst and (c) poly(lactide-*co*-alanine) using $Sn(OCt)_2$ as catalyst, at 195°C for 1 h (numbers correspond to integration of the spectra peaks).

^{1}H NMR ($CDCl_3$) of poly(lactide-*co*-glycine) (mole ratio of: L-lactide:glycine anhydride = 4:6, and mol ratio of comonomer:t-BuOK = 50:1 at 195°C for 1 h) δ= 1.6 (d, 3H, -CH-C<u>H</u>$_3$), 3.8 (b, 2H, -NH-C<u>H</u>$_2$), 4.4 (s, 1H, -CH_2-N<u>H</u>), and 5.2 (b, 1H, –O-C<u>H</u>-CH_3) (Fig. 1b); DSC: melting point due to polylactide blocks at 160.5°C; GPC: $\overline{M}_n$= 1450, $\overline{M}_w/\overline{M}_n$ = 2.7.

^{1}H NMR ($CDCl_3$) of poly(lactide-*co*-alanine) (mole ratio of: L-lactide:alanine anhydride = 4:6, and mole ratio of comonomer:$Sn(OCt)_2$ = 1000:1 at 195°C for 1 h) δ= 1.6 (d, 6H, -CH-C<u>H</u>$_3$), 4.4 (s, 1H, -CH-N<u>H</u>) and 5.2 (b, 1H, -O–C<u>H</u>-CH_3; 1H, -NH–C<u>H</u>-CH_3) (Fig. 1c); DSC: melting point due to polylactide blocks at 144.7°C; GPC : $\overline{M}_n$= 5130, $\overline{M}_w/\overline{M}_n$ = 1.6.

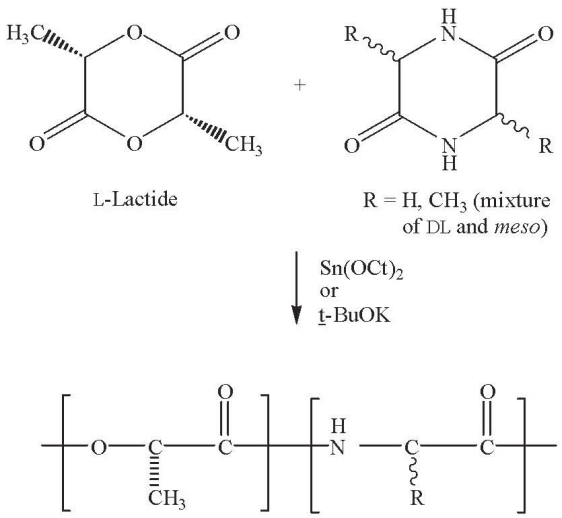
Biodegradability Assay of the Poly (Ester Amide)s

Poly(lactide-*co*-glycine) and poly(lactide-*co*-alanine) obtained were ground and passed through a 250 μm wire screen. Biodegradability of the poly(lactide-*co*-glycine) and poly(lactide-*co*-alanine) was assayed from the activity of solubilization using proteinase K by following the method previously reported[12]. The reaction was performed in a 50 ml vial containing 10 mg of powdered poly(lactide-*co*-glycine) or poly(lactide-*co*-alanine), 4.0 ml of 0.1 M phosphate buffer (pH 7.0), 0.1 ml of 0.5 wt./vol.% octyl glucopyranoside, and 0.1 mg (30 U/g protein) of product in a total volume of 5.0 ml. One unit (U) was defined as the amount of enzyme which formed 1 μmol of proteinase K in 1 min (pH 7.5, 35°C). In the substrate and enzyme controls, the enzyme and the substrate were omitted from the reaction mixture, respectively. After shaking the reaction mixture at 100 rpm, at 30°C for 12 h, the water soluble total organic carbon (TOC) concentration was measured. The TOC value of poly(lactide-*co*-glycine) or poly(lactide-*co*-alanine) was obtained by subtraction from both controls.

The biodegradability assay of poly(L-lactide, PLLA) was also carried out with the same procedure mentioned above to compare the activity towards the above copolymer.

Results and Discussion

Ring-opening copolymerization of L-lactide and glycine anhydride or alanine anhydride was first carried out by using tin(II) bis-ethylhexanoate ($Sn(OCt)_2$), the most commonly used catalyst in the ring-opening polymerization of L-lactide[22], as shown in Scheme 1. $Sn(OCt)_2$ was chosen because of its high catalytic activity and ability to give high molecular weight polymers with low racemization. Two amino acid anhydrides, glycine anhydride and alanine anhydride, were used because glycine and alanine are the two main amino acid components in silk fibroin and their degradability has been observed[4]. Preparation of biodegradable poly(ester-amide)s containing L-lactide and these amino acids was investigated. Copolymerize of L-lactide with glycine anhydride or alanine anhydride with the mole ratio of L-lactide:glycine anhydride or alanine anhydride = 9:1, 6:4 and 4:6 and the mole ratio of comonomer:catalyst = 50:1, 150:1, 500:1 were carried out at 130°C. At this temperature, $Sn(OCt)_2$ dissolves readily in the molten L-lactide, which ensures homogeneous catalysis. Attempts to copolymerize L-lactide with glycine anhydride or alanine anhydride by $Sn(OCt)_2$ at 130°C for 5 days were unsuccessful and could not be achieved when the temperature was increased to 170°C for 2 days. It had been reported that ring-opening of morpholine-2,5-dione proceeded through the cleavage of the ester group, not through the cleavage of the amide group, therefore ring-opening the amino acid anhydrides can be considered as an entropy-driven unfavorable process, so a much stronger condition was needed[23-25]. When the temperature was further increased to 195°C and the mole ratio of L-lactide:glycine or alanine anhydride = 4:6 with comonomer:catalyst mole ratio as high as 1000:1 was used. Calculation of integration obtained from NMR analysis showed that only 5 mol% of alanine with 34.7% yield was detected in the poly(lactide-*co*-alanine). However, relatively low molecular weight poly(lactide-*co*-alanine) was obtained ($\overline{M}_n$= 5130). Polymerization of lactides is usually associated with transesterification reactions at high catalyst amounts and high polymerization temperatures (>180°C). During transesterification or ester interchange



Scheme 1. Ring-opening polymerization of L-lactide and glycine anhydride or alanine anhydride (99% of DL and *meso*) using $Sn(OCt)_2$ as catalyst.

reactions, there are two ways in which the ester linkages between successive lactide units can cleave and reform. One is acyl-oxygen cleavage, which does not involve the chiral carbon, and the other is alkyl-oxygen cleavage, in which the covalent bond between oxygen and the chiral carbon breaks and subsequently reforms with an inversion of the configuration. High catalytic amounts result in an increase in the number of sites susceptible to ester interchange reactions, while high reaction temperatures favor the breaking of alkyl oxygen bonds, which leads to racemization. It was found that the risk of racemization even at high temperatures was significantly reduced due to the covalent nature of $Sn(OCt)_2$ initiator[26-27]. This aspect is important for the production of polylactides, because a final reaction temperature ≥195°C is needed.

Poly(lactide-*co*-glycine) and poly(lactide-*co*-alanine) could not be obtained when mole ratio of L-lactide and glycine anhydride or alanine anhydride = 6:4 and 9:1 were used. This was in agreement with previously reported results, that the formation of peptide bonds of triglycine, tetraglycine or pentaglycine from glycine anhydride and glycine, diglycine or triglycine respectively (glycine anhydride + $(glycine)_n \rightarrow (glycine)_{n+2}$) proceed through the nucleophilic attack of an amino group of the amino acids or the oligoglycines on the glycine anhydride accompanied by the ring-opening, thus the larger the number of *n*, the higher the yield of the resulting peptide[28].

Copolymerization of L-lactide with glycine anhydride was further studied using other catalysts. Because glycine anhydride is a weak base, a much stronger base than glycine anhydride is needed to open and polymerize glycine anhydride. Dibutyltin(IV) diacetate, a nonionic catalyst like $Sn(OCt)_2$, and t-BuOK, a stronger base than $Sn(OCt)_2$, commonly used in anionic ring-opening polymerization of cyclic amides or lactams for preparation of polyamides[29-31] or polypeptides[12], were investigated. Previous studies reported that an initiator such as t-BuOK rapidly initiates lactide polymerization, but side reactions, such as deprotonation, causing racemization of the lactide monomer, are also induced[32]. It was found that ring-opening copolymerization could not be obtained when dibutyltin(IV) diacetate was employed, while poly(lactide-*co*-glycine) with 5.7 mol% of glycine was detected at 25.4% yield, as calculated from integration of the NMR peaks, with t-BuOK as catalyst, the mol ratio of L-lactide:glycine anhydride = 4:6, and comonomer:catalyst = 50:1 at 195°C for 1 h. However, very low molecular weight poly(lactide-*co*-glycine) was obtained ($\overline{M}_n$ = 1450). This may be due to the cleavage of the ester linkages in poly(lactide-*co*-glycine) by t-BuOK.

It was found that proteinase K can degrade PLLA and poly(ester amide)s derived from L-alanine[20], thus the susceptibility of poly(lactide-*co*-glycine) and poly(lactide-*co*-alanine) to enzymatic hydrolysis by proteinase K was studied. A TOC (total organic carbon) analyzer was used to determine the degradability of both copolymers, since TOC analysis is a simple assay of released water-soluble degraded substances (monomer and oligomers) in enzymatic degradation mixtures. This enzymatic assay system did not necessarily involve complete degradation into the constituent units. The biodegradability assay of the copolymers was also simultaneously run on poly(L-lactide) (PLLA). It was found that the poly(lactide-*co*-glycine) and poly(lactide-*co*-alanine) can be degraded by proteinase K and the biodegradability decreased with increasing amino acid content (Table 1). This was in agreement with the previously reported results, which indicated that a high number the hydrogen bonding of amide linkages in poly(ester-amide)s had a strong effect on the biodegradation[12,16,18]. It was also confirmed that partial inclusion of glycine and alanine in L-lactide chains by ring-opening polymerization of L-lactide and amino acid anhydrides was obtained.

Table 1. Degradation of poly(lactide-*co*-glycine) and poly(lactide-*co*-alanine) by proteinase K.*

	TOC (ppm)		
	poly (lactide-*co*-glycine)	poly (lactide-*co*-alanine)	poly (L-lactide)
sample	115	192	316
substrate control	37	67	32

* Enzyme control TOC content = 39 ppm.

Conclusion

Ring-opening polymerization of L-lactide with amino acid anhydrides:glycine and alanine anhydrides was possible with t-BuOK and $Sn(OCt)_2$ as catalysts for synthesis of poly(lactide-*co*-glycine) and poly(lactide-*co*-alanine), respectively at 195°C for 1 h. However, the poly(lactide-*co*-glycine) ($\overline{M}_n$ =1450) and poly(lactide-*co*-alanine) ($\overline{M}_n$=5130) obtained had relatively low molecular weights. Studies on enzymatic hydrolysis showed that both copolymers were susceptible to degradation by proteinase K and also indicated that the derived poly(ester-amide)s have low levels of inclusion of glycine and alanine in the L-lactide chains. Further studies to investigate the possibility of other catalysts to promote ring-opening polymerization of both L-lactide and amino acid anhydrides are needed in order to increase the amide inclusion and the molecular weight of these poly(ester amide)s.

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