Biodegradable Polymers Derived From Aminoacids

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SUMMARY: Poly(amide-ester)s derived from five α-amino acid mixtures including glycine, DL- and L-alanines, DL- and L-phenylalanines, and three different diols including 1,6-hexanediol, 1,4-butanediol and *trans*-1,4-cyclohexanedimethanol were synthesized by interfacial, solution and melt polymerizations. All of the polymers had Tg's ranging from -6 to 50°C. The incorporation of rigid *trans*-1,4-cyclohexanedimethanol in the main chain significantly increased the Tg of these polymers. The degree of crystallinity depended on the type of amino acid and decreased with the size of substituent on the α-carbon in the amino acid. Biodegradation of these polymers were tested semi-quantitatively by turbidity measurements. Enzymes used included subtilisin, pronase E, α-chymotrypsin, *fusarium*, and lipase. The incorporation of *trans*-1,4-cyclohexanedimethanol unit slowed down degradation rate. Polymers containing L-amino acid generally degraded faster than the polymers containing DL-amino acids. Quantitative biodegradation testings using ninhydrin analysis, total organic analysis, and weight loss done on alanine derived polymers indicated that the degradation of the polymers by pronase E occurred at the ester bonds first and was specific against L-amino acid. The degradation was followed by slower amide bond degradation.

Introduction

The use of biodegradable polymer in biomedical applications and waste management have increased steady in recent years[1-16]. By and large aliphatic polyesters prepared by ring opening polymerization of cyclic lactones, such as glycolide, lactide, and to a much small extent trimethylene carbonate and dioxanone. The advance of the field is seriously material limited. We have directed some of our efforts toward the design, synthesis, characterization and testing of novel biodegradable polymers other than polyesters that are suitable for in vivo applications. In order to minimize potential toxicity of polymers and their degradation products we limited our choice of starting materials to natural

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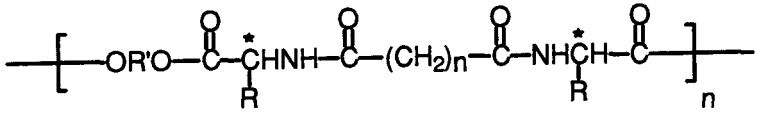

n = 8 ← From sebacoyl chloride

R' = $((CH_2)_x$, x = 4 or 6) or

(trans) ← From diols

R = H (Glycine)
or CH_3 (DL & L-Alanine)
or CH_2Ph (DL & L-Phenylalanine) ← From amino acids

• = Chiral center

Figure 1. General Structure of Poly(amide-ester)s and the Sources of the Structural Units

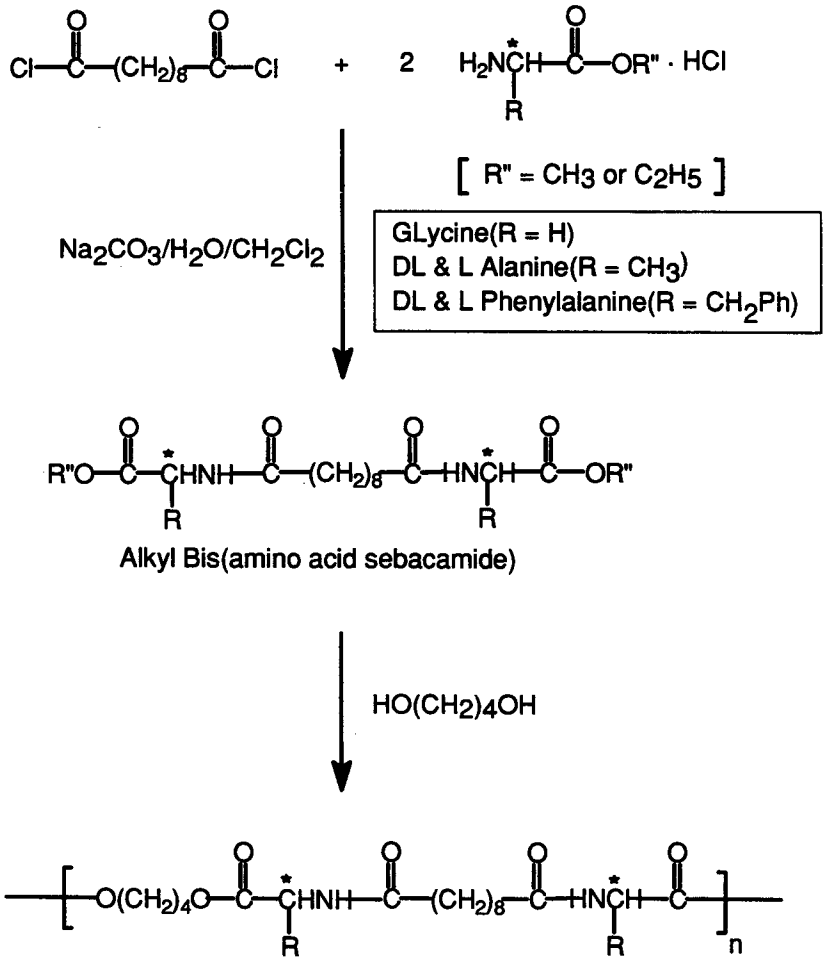

Figure 2. Synthesis of Poly(amide-ester)s by Melt Polymerization

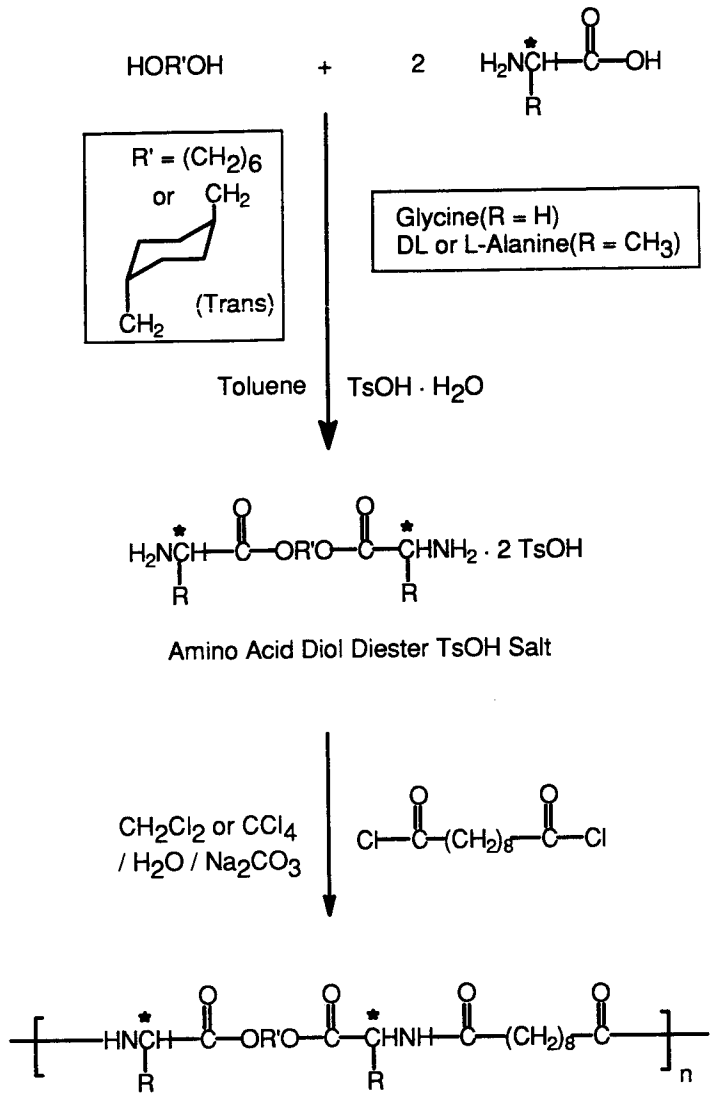

Figure 3. Synthesis of Poly(amide-ester)s by Interfacial Polymerization

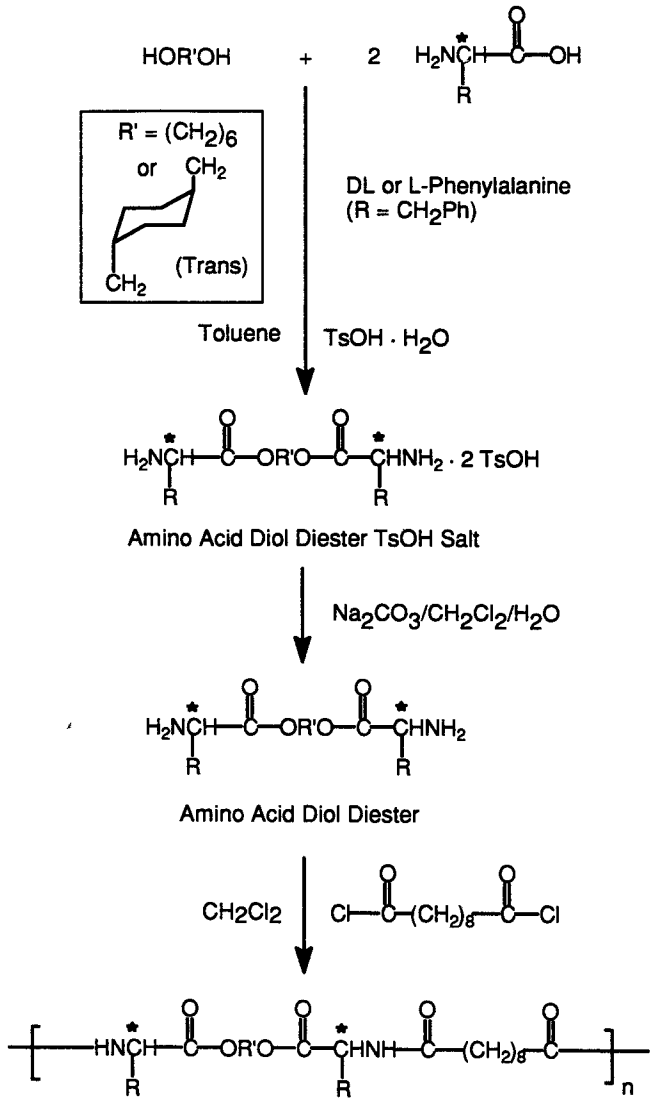

Figure 4. Synthesis of Poly(amide-ester)s by Solution Polymerization

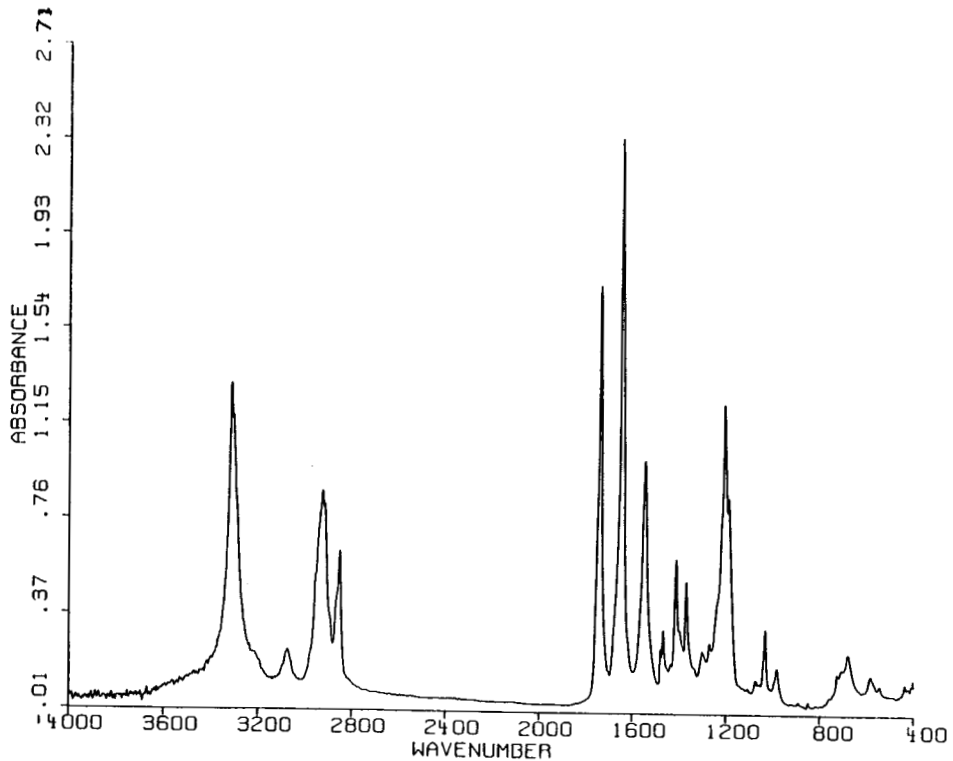

Figure 5. **FT-IR Spectrum of GHS Polymer**

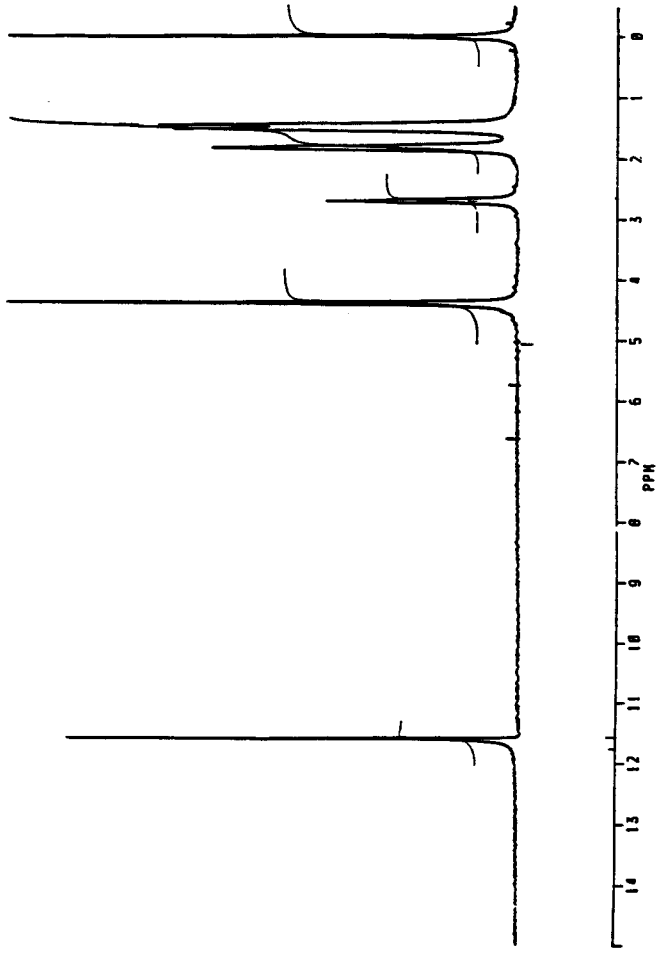

Figure 6. ^1H NMR Spectrum of GHS Polymer

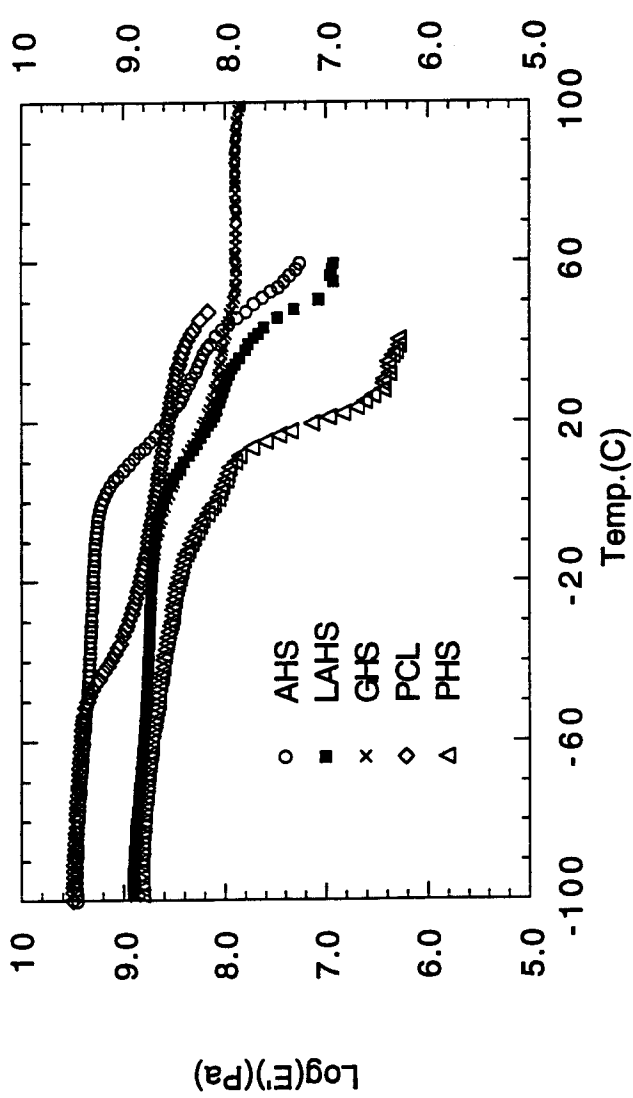

Figure 7. Storage Modulus vs. Temperature Plots for Poly(amide-ester)s as Measured by DMTA

Table 1. Summary of the Thermal Properties of Poly(amide-ester)s

Polymer	Tm(°C)	ΔHf(J/g)	Tg(°C)
Nylon 12/10	197(189&200)	86(72)	---(---)
Model Polymer 2[b]	156(157)	68(45)	---(---)
GHS	129&142(132&142)	83(49)	---(---)
AHS[c]	40&90(---)	40(---)	---(19)
LAHS[c]	38&86(---)	48(---)	---(8)
PHS	---(---)	---(---)	27(27)
LPHS	---(---)	---(---)	5(5)
GCHS	173(176)	53(53)	---(---)
ACHS	96(---)	36(---)	(50)
LACHS	103&124(---)	35(---)	(47)
PCHS	---(---)	---(---)	49(49)
LPCHS	127&137(---)	30(---)	42(47)
GBS	139&154(135&154)	84(73)	---(---)
ABS	105&112(111)	57(44[d])	---(4)
LABS	93&101(---)	56(---)	---(7)
PBS	---(---)	---(---)	8(7)
LPBS	---(---)	---(---)	(-6)
(G-co-P)HS	55(---)	12(---)	(4)
(G-co-LP)CHS	89(---)	16(---)	37(45)

a: Data in parenthesis were obtained from the 2nd heats after quenching; b: Poly(4,9-dioxa-1,12-dodecamethylene sebacamide); c: Data obtained from the polymer annealed at 56°C for 4 days; d: This was due to the melting of the recrystallization of the polymer

products and synthetic compounds that are known to be assimilable by the body. Saccharides, poly saccharides, fatty acids, α-aminoacids, and glycols are among those we have used since they are in general biocompatible. Alpha-aminoacids are of special interest to us and other researchers since they provide a large variety of structural feature that can be incorporated in the polymer chains resulting in aboard spectrum of property and degradability[7-11]. As part of our systematic studies of the effect of structural feature on biodegradability we have studied multi-functional group containing polymers [12-14]. We report our recent results on poly(amide-ester)s derived from alpha-aminoacids.

Results and Discussion

Conformational flexibility of polymer chains facilities biodegradation. As a result aliphatic polyesters are the main group of polymers that are practical biodegradable polymers. Acids are generated during the degradation of polyesters and in cases where surface/volume ratio is small autocatalysis occurs[16]. We reasoned that hydrolysis of the amide group of poly(amide-ester)s would produce amines and acids which should act as buffers resulting less change of pH as compared with polyester hydrolysis. Convenient synthetic procedures were used for the preparation of poly(amide-ester)s including the synthesis of aminoacid glycol diesters and diesters of aminoacid dicarboxylates as monomers; interfacial, solution and melt polymerization to give the poly(amide-ester)s. Structures of the polymers prepared for synthesis are shown in Figures 2-4. These polymers have molecular weight ranging from 10,000-35,000 (by size exclusion chromatrograp, end group analyses and in some cases, N.M.R.). FTIR, N,M.R. and elemental analyses supported the assigned structures[15]. Representative spectra are shown in Figure 5 & 6 for glycine derived poly(amide-ester), GHS. Depending on the substituent and optical composition of the alpha-aminoacids used the resulting polymers are mostly partial crystalline with exception of some polymers derived from phenylalanine, which remain amorphous after aging several months at room temperature, Table 1. The melting points are lower that those of analogous polyamides, Nylon 12, 10 at 197^{0}C, poly(4,9-dioxa-1,12-dodecamethylene sebacamide) at 156^{0}C and GHS at 129 & 142^{0}C. These polymers have the same sebacyl residue but amine residue of different flexibility. Preliminary degradation results also indicate that the rate of degradation increases with flexibility. Interestingly all these polymers are highly crystalline (high ΔH_f) and DSC studies could not detect the Tg's. However, DMTA studies, Figures 7 & 8, do reveal Tg's generally below r.t. as expected due to the ester segments.

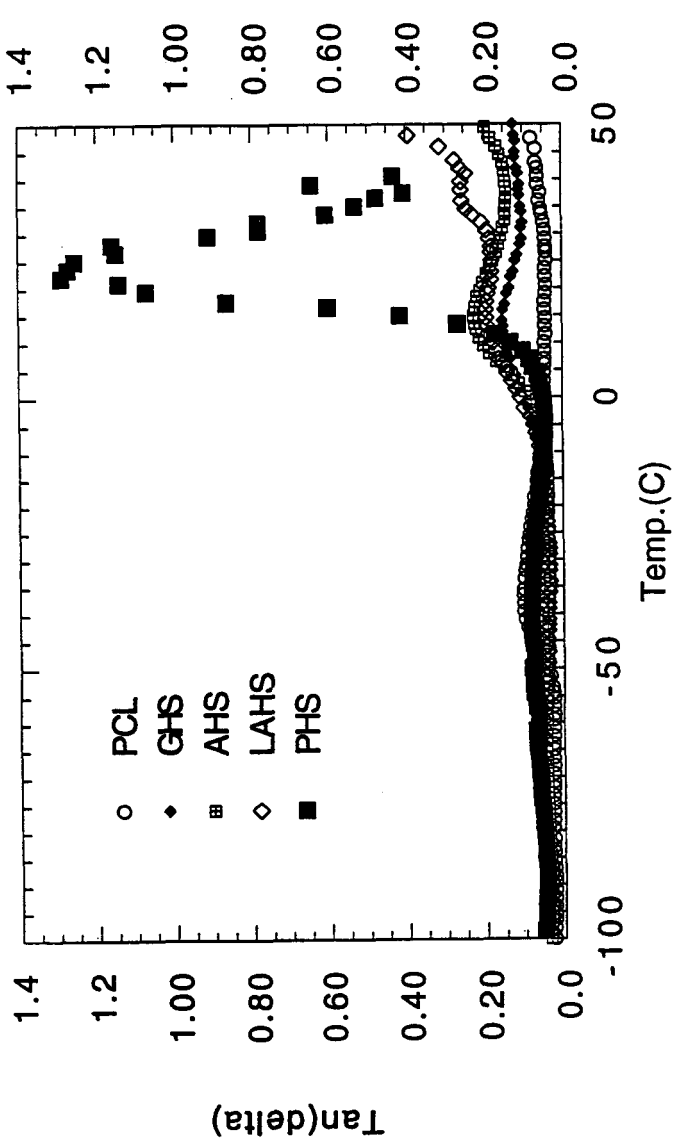

Figure 8. Tanδ vs. Temperature Plots for Poly(amide-ester)s

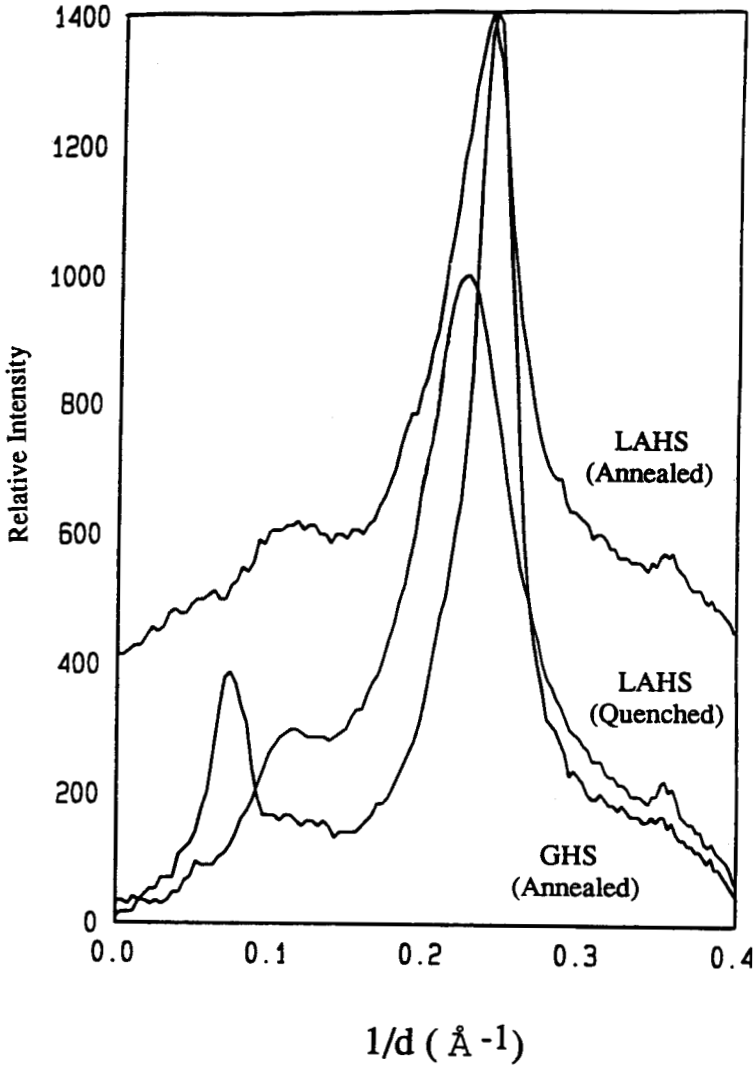

Figure 9. Relative Intensity Profiles of the Small Angle x-Ray Scattering Patterns for LAHS and GHS Polymers

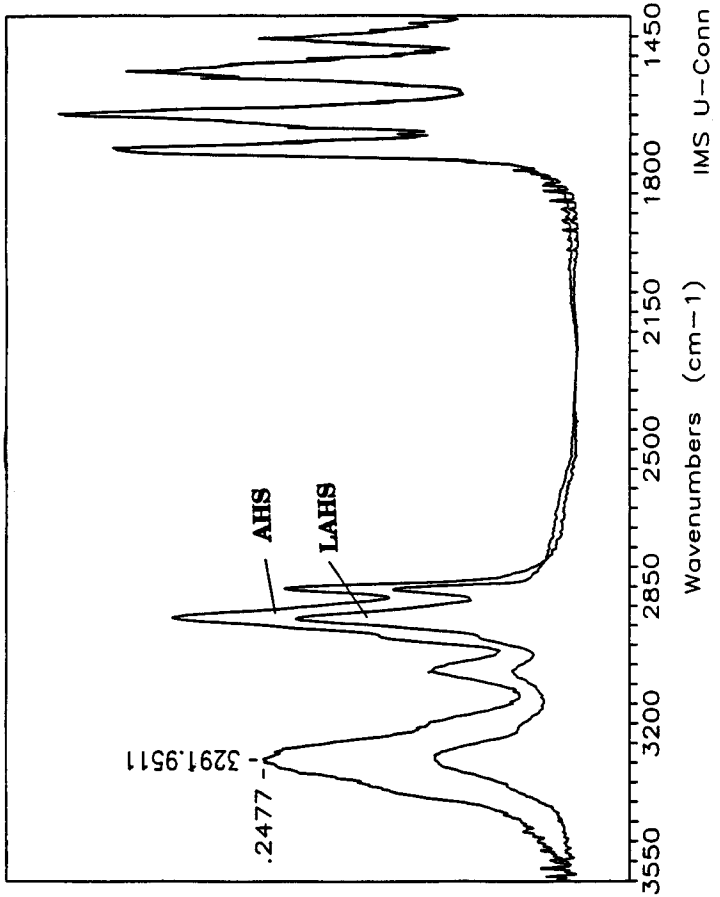

Figure 10. FT-IR Spectra for AHS and LAHS Polymers

Table 2. Summary of the Enzyme Degradation of Poly(amide-ester)s as Measured by Turbidity Measurements.

Polymer	Subtilisin	*Fusarium*	Lipase	Pronase E	a-Chymotrypsin
PCL(DMF)	1.0	4.0	6.0	0.0	1.3
PCL(THF)	2.0	35.3	0.67	0.0	0.0
GHS	0.0		0.0	0.0	0.0
AHS	12.7		0.7	10.7	5.3
LAHS	8.7	0.0	4.0	32.7	1.3
PHS	0.7		0.7	0.0	0.0
LPHS	3.3		10.7	3.3	13.3
GCHS	0.0		0.0	0.0	0.0
ACHS	1.3	1.3	0.0	2.7	1.3
LACHS	0.0		2.7	1.3	3.3
PCHS	0.0		1.3	2.0	1.3
LPCHS	0.0		6.0	1.3	0.0
GBS	0.0	0.0	1.3	0.0	0.0
ABS	1.3	2.7	0.7	3.3	0.7
LABS	62.7		2.7	28.7	6.7
PBS	4.7	0.0	12.7	0.0	31.3
LPBS	8.0	0.0	14.7	0.0	40.0

Unit = (Δ O.D./(min/mg enzyme))*1000

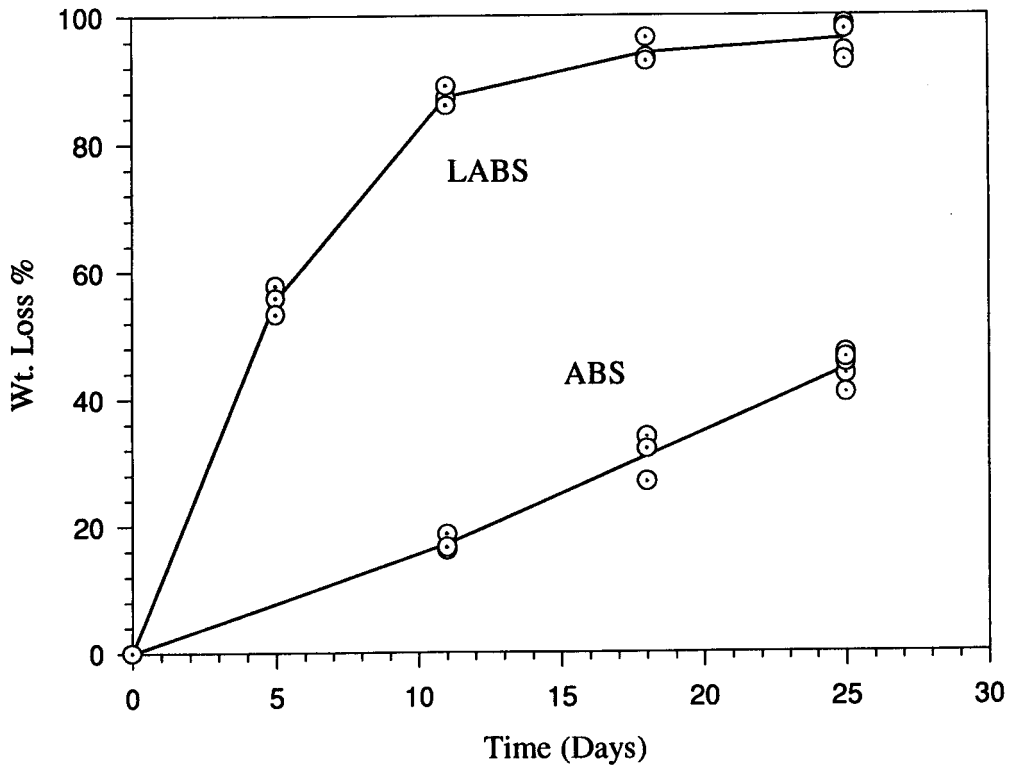

Figure 11. Weight Loss of ABS and LABS Polymers by Pronase E Hydrolysis

Phenylalanine derived polymers are tough materials as indicated by Tan δ, Figure 8. Annealing increases the crystalinity with the optical active monomers derived one better than the one from racemic monomers, as shown by small angle x-ray scattering pattern, Figure 9. It is interesting to note that the "racemic" poly(amide-ester)s have greater extent of H-bonding than the "chiral" poly (amide-esters) as shown by FTIR of LAHS and AHS, Figure 10. This also reflect in higher Tg for the "racemic" polymers that the "chiral" polymers. The arrangement of the structural units in these poly(amide-ester)s are "head-to-head" as compared with "head-to-tail" for poly peptides and poly hydroxycarboxylates, Figure 12. The effect of this on the physical and chemical properties is a subject of our continuing study. As expected, the degradation by enzymes are substituent and chirality specific, Table 2, where semi-qualitative tubidity measurements were used to compare the trends. For example, alpha-chymotoypsin degrades phenylalanine derived PHS and LPHS. This agrees with the result of Eudo et. al. [11]. Subtilisin and pronase E are active toward poly(amide-ester)s, specially the chiral LAHS, Figure 11.

Experimental

General Characterization Procedures

Fourier Transform Infrared spectra (FT-IR) were recorded on a Nicolet model 60SX spectrophotometr at a resolution of 4 cm^{-1}. Sample preparation involved either casting solution onto KBr disk to form a film, or blending and grounding with KBr powder then pressing into a KBr pellet. Solution FT-IR spectra were recorded between two KBr windows using a rectangular Teflon spacer sandwiched in between. Solvent spectra were used as the background for each spectrum. Sample spectra were then recorded and ratioed against the background. Abbreviations used for FT-IR spectra data are as follow: g=stretching mode; d=bending mode or deformation; max=maximum intensity; Ar=aromatic. Fourier Transform Nuclear Magnetic Resonance spectra (FT-NMR) were recorded on a Bruker AC270 Spectrophotometer. Tetramethysilane was used as the internal standard. All sample solutions were filtered before measurements to assure homogeneity. Abbreviations used for FT-NMR spectra data are as follow: s=singlet; d=doublet; t=triplet; q=quadruplet; m=multiplet; dd=doublet of doublet. Differential scanning calorimetry (DSC) as performed on a Perkin-Elmer were carried out after samples had been quenched form the melt using a fast cooling rate (200^{0}c/min). The instrument was calibrated by and indium standard. Solubility

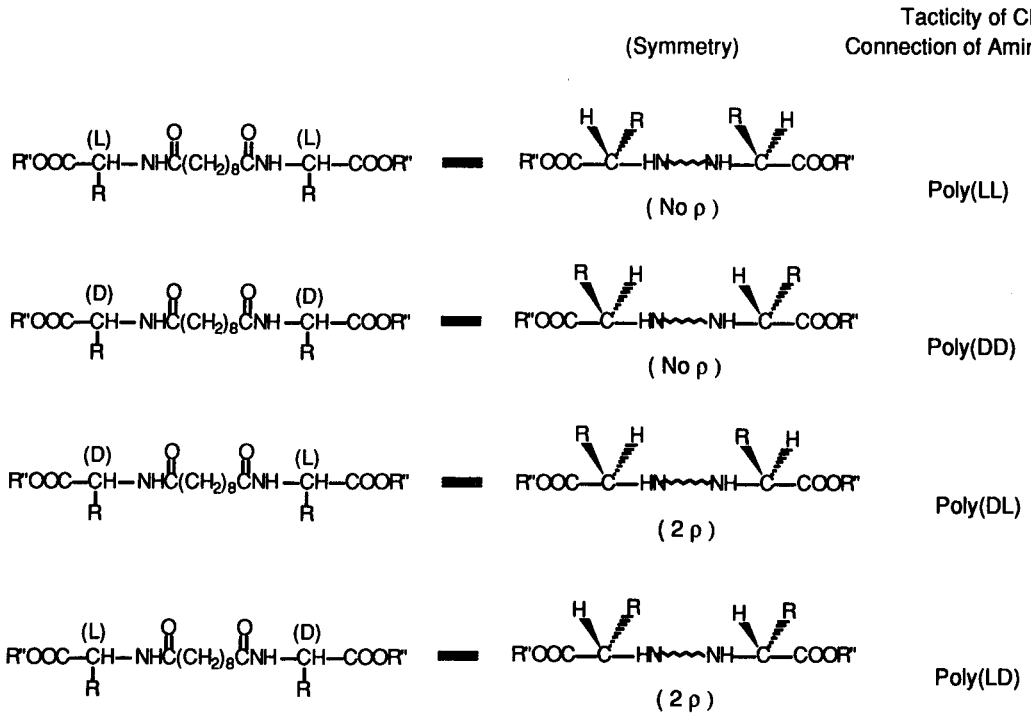

Figure 12. Possible Connections of the Amino Acid Units in Alkyl Bis(DL-amino acid sebacamide)

tests of the polymers were carried out using a concentration of 0.1 w/v (solute/solvent). For samples using low-boiling solvents such as chloroform ($CHC1_3$), methylene chloride (CH_2C1_2) and tetrahyddrofuran (THF), testing was done at RT. For the tests using high-boiling solvents, such as N,N-dimethylformamide (DMF), they were done both at RT and 70°C. The mixtures of polymer and solvent were heated and maintained at 70°C to determine their solubilities at 70°C. These mixtures were then cooled to RT to determine their solubilities at RT. Dynamic Mechanical Thermal Analysis (DMTA) was performed on a Polymer Laboratories DMTA MKII outfitted with a dual-cantilever head, using a heating rate of 5°C/min. Frequency used was 1 Hz. Samples used for the measurements were compression molded in between two Teflon-coated polyimides films, and quenched in between two cold steel plates. The molding temperatures wer 160°C for GHS polymer, 120°C for both AHS and LAHS polymers, and 70°c for PHS polymer. Since AHS, LAHS and PHS polymers had low degree of crystallinity at RT, the soft films obtained from the molding processes were put in between two polyimide films and glass plates with a spacer in between, and annealed at RT for at least 2 weeks before used for DMTA measurements. Small angle x-ray scattering was carried out using Cu-Kα radiation (λ=1.54 Å). The diffractio patterns obtained were transformed into relative intensity profiles. The quenched AHS and LAHS polymers were prepared in a manner similar to those used for the DMTA measurements. without the annealing process. The annealed GHS, AHS and LAHS polymers the same samples but annealed at RT for at least 6 months.

Solvents and Reagents

All chemicals were obtained as reagent grade materials and used as received unless specified. 1,4-butanediol was purified by refluxing, then vacuum-distilling from sodium. Distilled triethylamine was distilled from calcium hydride under an Ar atmosphere. Distilled sebacoyl chloride was obtained by vacuum distillation and kept under Ar before use. Dry methylene chloride and toluene were obtained by refluxing, then distilling from calcium hydride. Activated carbon was obtained from Aldrich Chemical Co. Inc. (cat.#26001-0) and used as received.

Syntheses

All monomers and polymers synthesized were characterized with spectroscopic, molecular weight and elemental analyses. Details were reported elsewhere[15].

Monomer Synthesis

(A) Amino Acid Diol Diester TsOH Salts

The first type of monomers was synthesized from the reaction of various diols and amino acids in the presence of toluene and TsOH. Typical synthetic procedure is as follows. In a 3-neck, round-bottom flask equipped with an Ar inlet, a magnetic stirrer, a condenser with a calcium sulfate drying tube, and a Dean-Stark trap was charged with 1,6-hexanediol or 1,4-*trans*-cyclohexanedimethanol, amino acid (2% in excess relative to the diol), TsOH monohydrate (6% in excess relative to the diol), and toluene (c.a. 100 ml per 100 ml of amino acid). The reaction mixture was refluxed until no more water was distilled out of the mixture. On cooling to RT, the white or pale yellow, viscous liquid gradually solidified. Most of the toluene was decanted and the solid was vacuum dried at 70-100^{0}C overnight to get either a white or pale yellow solid.

(B) Amino Acid Diol Diesters

This type of monomers was used primarily to precisely control the stoichiometric balance needed for solution polymerizations which were carried out by reacting these monomers with sebacoyl chloride in methylene chloride. All the amino acid diol diesters were used to synthesize polymers within a day of their preparation in order to avoid any possible oxidation. Typical synthetic procedure is as follows. A two liter beaker was charged with amino acid diol diester TsOH salt, anhydrous potassium carbonate (10% in excess), methylene chloride (c.a. 2 ml per 1 mmol of amino acid diol diester TsOH salt), andd distilled water (same amount as methylene chloride). The mixture was stirred for at least 2 hours. The organic layer was then separated using a separatory funnel. The water layer was washed twice with methylene chloride. All of the methylene chloride layers were combined, dried over magnesium sulfate and then evaporated to dryness. After vacuum drying at RT overnight, the monomer was obtained as a pale yellow liquid.

(C) Alkyl Bis-(amino acid sebacamide)s

This type of monomers was synthesized by interfacial reaction between amino acid alkyl ester hydrochloride in the aqueous phase, containing a weak base, and sebacoyl chloride in methylene chloride. The alkyl group on the amino acid ester was either methyl or ethyl, depending on their availability. A small excess amount of amino acid alkyl ester hydrochloride against the amount of sebacoyl chloride was used to make sure that both acyl chloride groups in the sebacoyl chloride was used to make sure that both acyl chloride groups in the sebacoyl chloride reacted. Similarly, a small excess amount of weak base was used, against the amount of amino acid alkyl ester hydorchloride, to make sure all of the amino acid alkyl ester hydrochloride was freed from the hydrochloride ad available for the reaction. Typical synthetic procedure for this type of monomers is as follows. A commercial blender was charged with amino acid alkyl ester hydrochloride (2% in excess relative to sebacoyl chloride) and anhydrous potassium carbonate (2% in excess relative to amino acid alkyl ester hydrochloride). Methylene chloride (c.a. 1.5 ml per 1.00 mmol of alkyl ester hydrochloride) and sebacoyl chloride were then added. Distilled water (same amount as methylene chloride) was added immediately and the blender was stirred at low speed for one to two minutes. The methylene chloride layer was separated. The water layer was then washed twice with small amount of methylene chloride. All of the methylene chloride layers were combined and washed with dilute aqueous potassium carbonate, then distilled water. This interfacial raction was repeated several times to get enough amounts of monomer. All of the methylene chloride layers from the repeated reactions were combined, dried over magnesium sulfate, then evaporatd to dryness to obtain a white or pale yellow powder. Small amount of acid impurities were removed by either treating the powder with activated carbon in hot acetone, filtering, then recrystallizing from acetone/hexanes (glycine and phenylalanine-containing monomers), or by column chromatography using aluminum oxide as the stationary phase and acetone as the moving phase (alanine-containing monomers). After vacuum drying, the monomers were obtained as needle-like crystals (glycine), white powder (DL-and L-alanine) or pale yellow powder (DL-and L-phenylalanine).

Polymer Synthesis

(A) Interfacial Polymerizations

The following procedure used to synthesize GHS polymer was treated as the standard interfacial polymerization procedure for the preparation of all other polymers synthesized by interfacial polymerization.

Poly(oxyhexamethyleneoxycarbonylmethyleneiminocarbonylocta-methylenecarbonyliminomethylenecarbonyl), GHS

In a commercial blender was charged with 54.5 g (94.5 mmol) of glycine 1,6-hexanediol diester TsOH salt and 2 liters of distilled ice water. 40.9 g of (385.6 mmol) of anhydrous sodium carbonate was added and stirred at low speed for 30 seconds. The mixture turned into a clear solution. A mixture of 500 ml of methylene chloride and 19.35 ml (90.7 mmol) of distilled sebacoyl chloride was added. The container used to hold the distilled sebacoyl chloride and methylene chloride was rinsed with 100 ml of methylene chloride and the solution was added into the blender. The entire mixture was stirred at low speed for two to three minutes. The solid white powder formed from the reaction was filtered, washed with distilled water, acetone, and then vacuum dried at 80^{0}C to get 33.07 g (88%) of white powder.

(B) Solution Polymerizations

The following procedure described for the preparation of PHS polymer was treated as the standard procedure for the preparation of all other polymers synthesized by solution polymerization.

Poly(oxyhexamethyleneoxycarbonyl-DL-benzylideneiminocarbonylocta-methylenecarbonylocta-methylenecarbonylimino-DL-benzylidenecarbonyl), PHS

In an Ar-filled glove box, 16.490 g (39.97 mmol) of DL-Phenylalanine 1,6-hexanediol diester was weighed into a 500 ml round bottom flask. Another 250 ml round bottom flask was charged with 9.559 g (39.97 mmol) of distilled sebacoyl chloride and 80 ml of dry methylene chloride. Both flasks were capped, then removed from the glove box. Under an Ar atmosphere, 11.67 ml (8372 mmol) of distilled triethylamine and 100 ml of dry methylene chloride were added to the first flask, then the flask was cooled in a water bath. The contents of the second flast were added

to the first flask. The second flask was washed with two portions of 10 ml dry methylene chloride and added to the first flask to effect complete transfer of the reagents. All of the transfers and washings were done under an Ar atmosphere. After the reaction mixture was stirred under Ar for 15 hours, it was poured gradually into 1000 ml of boiling water in a 2500 ml beaker. After the precipitation was completed, the water was drained and ice was added. The precipitated polymer was collected as an elastic material from the bottom of the beaker. It was further purified by dissolving in methylene chloride, and washing with water, then precipitating into boiling water. The polymer collected was vacuum dried at 80^0C to get a pale yellow, rubber-like material (20.07 g, 87%).

Polymers Synthesized

Poly (oxyhexamethyleneoxycarbony-DL-ethylideneiminocarbonylocta-methylenecarbonylimino-DL-ethylidenecarbonyl), AHS

Poly (oxyhexamethyleneoxycarbonyl-L-ethylideneiminocarbonyloctamethylenecarbonylimino-L-ethylidenecarbonyl), LAHS

Poly(oxyhexamethyleneoxycarbonyl-DL-benzylideneiminocarbonylocta-methylenecarbonliminomethylenecarbonyl), (G-*co*-P) HS

Poly (oxymethylene-*trans*-1,4-cyclohexylemenethyleneoxycarbonyl-methyleneiminocarbonlocta-methylenecarbonyliminomethylene-carbonyl), GCHS

Poly(oxymethylene-*trans*-1,4-cyclohexylenemethyleneoxy-carbonyl-DL-ethylideneiminocarbonyloctamethylenecarbonylimino-DL-ethylidene-carbonyl) ACHS

Poly(oxmethylene-*trans*-1,4-cyclohexylenemethyleneoxy-carbonyl-L-ethylideneiminocarbonyloctamethylenecarbonylimino-L-ethylidene-carbonyl), LACHS

Poly(oxymethylene-*trans*-1,4-cyclohexylenemethyleneoxy-carbonyl-L-benzylideneiminocarbonyloctamethylenecarbonyliminomethylene-carbonyl), (G-*co*-LP) CHS

Poly(oxymethylene-*trans*-1,4-cyclohexylenemethyleneoxy-carbonyl-L-benzylideneiminocarbonyloctamethylenecarbonyl-imino-L-benzylidenecarbonyl), LPCHS

Poly(oxyhexamethyleneoxycarbonyl-L-benzylideneiminocarbonylocta-methylenecarbonylimino-L-benzylidenecarbonyl), LPHS

Poly(oxytetramethyleneoxycarbonylmethyleneiminocarbonylocta-methylenecarbonyliminomethylecarbonyl), GBS

Poly(oxymethylene-*trans*-1,4-cyclohexylenemethyleneoxycarbonyl-DL-benzylideneiminocarbonylocta-methylenecarbonyl-imino-DL-benzylkidenecarbonyl, PCHS

Poly(oxytetramethyleneoxytrimethyleneiminocarbonyloctamethylenecarbonyiminotrimethylene), Poly(4,9-dioxa-1,12-dodecamethylene sebacamide)

Poly(iminododecamethyleneiminocarbonyloctamethylenecarbonyl), Nylon 12/10

Biodegration

Methods have been reported elsewhere[7, 13, 15].

Conclusion

Potentially very useful biodegradable poly(amide-ester)s of large variety of structute, property and degradability have been prepared from apha-amino acids, glycols and dicarboxylic acids.

Acknowledgement

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