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Novel Enzymatically Degradable Polymers Comprising α-Amino Acid, 1,2-Ethanediol, and Adipic Acid

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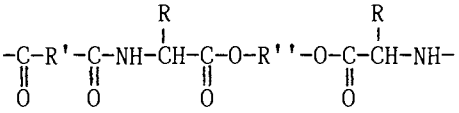
Novel water-insoluble polymers, which are degraded and solubilized by the action of specific proteolytic enzymes, were successfully synthesized from α-amino acid, 1,2-ethanediol, and adipic acid.

Many research efforts have been directed toward the synthesis of biodegradable polymers for applications in medical fields such as controlled drug delivery formulations and implant materials.[1] Although almost all the polymers of this class are degraded by hydrolysis not involving enzymatic catalysis, a few enzymatically degradable water-insoluble polymers have been synthesized by using α-amino acids[2] and their analogues.[3] We describe here the synthesis and enzymatic degradation of new polymers of this class comprising N-acyl-L-α-amino acid ester in their repeating backbone. In designing the polymers, we took it into consideration that degradable polymers to be used in the medical fields must be nontoxic, and that their metabolites must also satisfy this criterion.

It is well-known that the esters of N-acyl-L-α-amino acids are easily cleaved by the action of appropriate proteolytic enzymes. For example, trypsin hydrolyzes Nα-benzoyl-L-arginine ethyl ester to afford Nα-benzoyl-L-arginine so effectively that the reaction is used to assay the activity of the enzyme.[4] We therefore designed the degradable polymer whose main chain is composed of repeating N-acyl-α-amino acids:



where R is a side chain of amino acids and R' and R'' are derived from dicarboxylic acid and diol components, respectively. To meet the requirements of low toxicity, adipic acid

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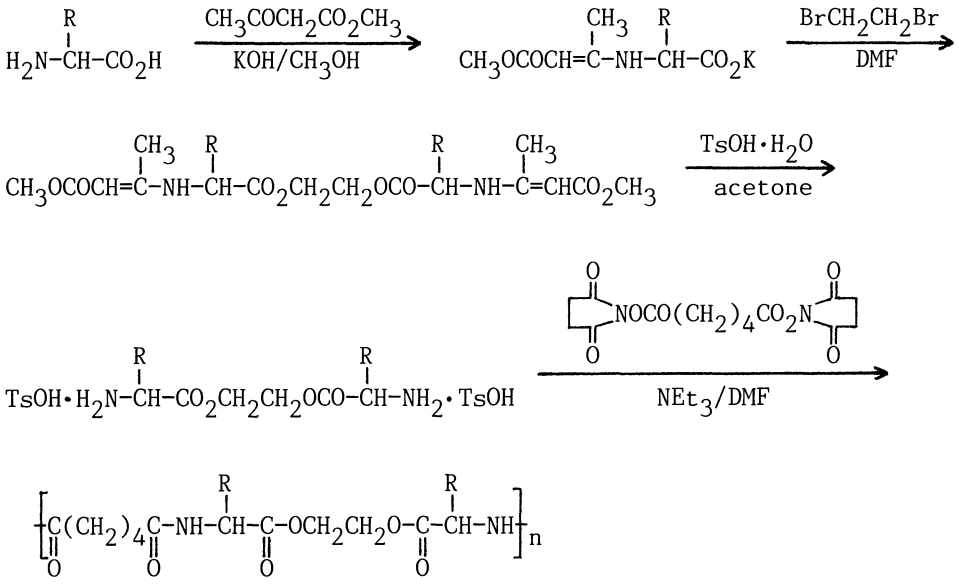
($R'=C_4H_8$) and 1,2-ethanediol ($R''=C_2H_4$) were employed here.

Glycine, L- and D-leucine, and L-, D-, and DL-phenylalanine were used as starting amino acids. At first, esterification by using Dane's enamine[5-7] as an N-protecting group, was performed to give six monomers, which were isolated as the dibasic salts of p-toluene sulfonic acid (TsOH).[8] Polymerization with disuccinimidyl adipate[9] was then carried out in N,N-dimethylformamide (DMF) at room temperature to yield water-insoluble polymers.[10] However, molecular weights for the obtained polymers could not be determined due to their insolubility in common organic solvents. Reaction scheme is summarized below.



We then carried out the degradation experiment of thus obtained polymers. The polymers were finely powdered and incubated at 30 °C with shaking either in the presence or in the absence of some common proteolytic enzymes under a buffered condition.[11] After 12 h, the total amount of soluble organic carbon (TOC) in the buffer solution was measured.[12] As shown in Table 1, the Gly-derived polymer was not degraded by any enzymes tested here, although it was degraded and solubilized to some extent even in the absence of enzymes. In contrast, the Phe-derived polymer was significantly degraded and solubilized by the action of chymotrypsin, which cleaves the C-terminal bond of aromatic amino acids in various peptides under physiological conditions.[13] The Phe-polymer was also degraded by elastase, and the Leu-derived polymer underwent degradation by chymotrypsin to some extent. In order to investigate whether thus observed degrading solubilization involves the normal catalytic action of an enzyme, the D-isomer of the Leu-derived polymer and the D- and DL-isomers of the Phe-polymer were examined by using chymotrypsin. As summarized in Table 2, the D-leucine- and D-phenylalanine-derived polymers did not undergo enzymatic solubilization at all, and it was further found that the DL-Phe-polymer was degraded and solubilized to a considerable extent. This clearly indicates that the degrading solubilization of the polymers has proceeded involving the normal catalytic site of chymotrypsin.

Chromatographic metabolite analysis showed that the Phe-derived polymer gave the 40% yield of the di(L-phenylalanine)amide of adipic acid after incubation with chymotrypsin for 12 h, whereas no phenylalanine itself was detected.[14] This means that the solubilization of the polymer is not caused by an amide bond hydrolysis but by that of an ester, which in turn adds further proof to the degradation mechanism discussed above.

Table 1. Hydrolysis of polymers by enzymes[a]

Enzyme	pH	TOC formed/ppm			
		Polymer from			
		Gly	Leu	Phe	(none)[b]
Pepsin[c]	2.0	152	126	97	120
(none)	2.0	88	14	13	-
Trypsin	7.6	299	199	267	161
(none)	7.6	118	15	21	-
Chymotrypsin	7.8	317	559	1482[d]	163
(none)	7.8	134	17	19	-
Esterase[c]	8.0	399	198	193	193
(none)	8.0	130	17	23	-
Elastase	8.8	390	300	1341	140
(none)	8.8	204	29	22	-

a) For testing procedure, see Ref. 11. b) The value is that of the enzyme used. c) Subtracting the control (no enzyme) and blank values (no polymer) makes the concentration less than zero, which might indicate that these enzymes tend to become insolubilized by the incubation with the polymers. d) The value at 3 h, 637 ppm; 6h, 1010 ppm; 9h, 1134 ppm.

Table 2. Effect of stereochemistry[a]

Polymer from	TOC formed/ppm	
	Chymotrypsin	
	Present	Absent
Leu	559	17
D-Leu	163	15
Phe	1482	19
DL-Phe	944	34
D-Phe	125	19
(none)	163	-

a) For procedure, see Ref. 11.

References

1) For a review, see, for example, G. S. Kumar, "Biodegradable Polymers," Marcel Dekker, New York (1987).
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7) General procedure for N-protected amino acid potassium salts. To a suspension of amino acid (100 mmol) in methanol (150 mL) was added 85% potassium hydroxide (6.60 g) in several portions at room temperature, followed by the addition of methyl acetoacetate (100 mmol). The reaction mixture was stirred for 3 h, and the resultant precipitates (in the case of Gly) or the residual solids after evaporation (Phe, Leu) were recrystallized from methanol (Gly, 96.4% yield, mp 241 °C), 2-propanol-hexane (Leu, 81.3%, 219 °C; D-Leu, 55.1%, 219 °C), or ethanol-diethyl ether (Phe, 81.4%, 209 °C; D-

Phe, 82.5%, 209 °C; DL-Phe, 93.9%, 174 °C).

8) General procedure for amino acid monomers. To a solution of 1,2-dibromoethane (25 mmol) in DMF (70 mL) was added 50 mmol of an N-protected amino acid potassium salt, and the reaction mixture was stirred at 50 °C for 5 h. After the addition of water (120 mL), the mixture was extracted with ethyl acetate (4 x 100 mL), washed with water (4 x 200 mL), dried over magnesium sulfate, and evaporated to dryness. To the residue was added acetone (200 mL), followed by p-toluene sulfonic acid monohydrate (50 mmol), and the solution was stirred for 30 min. After the addition of diethyl ether (200 mL), the white precipitates were collected by filtration and recrystallized from ethanol-diethyl ether (Gly, 61.0% yield, mp 154 °C; Leu, 92.8%, 238 °C; D-Leu, 87.7%, 238 °C), methanol-ethanol (Phe, 82.9%, 235 °C; D-Phe, 59.7%, 235 °C), or methanol-ethanol-diethyl ether (1:1:3) (DL-Phe, 57.4%, 252 °C). All the monomers gave satisfactory elemental analysis.
^{1}H NMR (DMSO-d_6) for the monomers. Gly-derived: δ 8.24(s, 6H), 7.49(d, J=8.0, 4H), 7.12(d, J=8.0, 4H), 4.40(s, 4H), 3.85(s, 4H), 2.30(s, 6H). Leu-derived: δ 8.35(s, 6H), 7.49(d, J=8.0, 4H), 7.12(d, J=8.0, 4H), 4.41(AB, 4H), 4.00(t, J=7.0, 2H), 2.29 (s, 6H), 1.78-1.71(m, 2H), 1.64-1.60(m, 4H), 0.90(d, J=6.5, 12H). Phe-derived: δ 8.44 (s, 6H), 7.50(d, J=8.0, 4H), 7.35-7.20(m, 10H), 7.13(d, J=8.0, 4H), 4.32-4.17(m, 6H), 3.14-3.03(m, 4H), 2.03(s, 6H).

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10) To a solution of an amino acid-derived monomer (1.000 mmol) and disuccinimidyl adipate (1.000 mmol) in DMF (2 mL) was added triethylamine (2.1 mmol) at room temperature. The solution was stirred for 12 h, and the resultant suspension was poured into water (100 mL). The white precipitates were collected and extensively washed either with methanol (Gly, 57% yield) or with ethanol-diethyl ether (1:3) (Leu, 60%; D-Leu, 61%; Phe, 69%; D-Phe, 84%; DL-Phe, 91%). All the polymers gave satisfactory elemental analysis.

11) All the enzymes used in this study were purchased from Sigma Chemical Co. Buffer solutions (50 mM) are either borate (pH 2.0 for porcine stomach mucosa pepsin A) or phosphate (pH 7.6 for porcine pancreas trypsin, 7.8 for bovine pancreas α-chymotrypsin, 8.0 for porcine liver esterase, 8.8 for porcine pancreas elastase). Polymer specimens were finely grounded to pass through a 100 μm sieve. Into a 12 mm i.d. glass tube containing 1.00 mg of an enzyme and 3.00 mL of an appropriate buffer was added the powdered polymer (10.0 mg). A tube deprived of an enzyme (a control) and a tube excluding a polymer (a blank) were also prepared. Incubation was conducted in a water bath at 30 °C with shaking at 75 strokes per minutes for 12 h. The reaction mixture was then filtered through a 0.22 μm membrane at 6500 rpm. The total amount of soluble organic carbon in the filtrate was determined on a Shimadzu TOC-500 analyzer.

12) Y. Tokiwa and T. Suzuki, Nature, 270, 76 (1977).

13) M. Laskowski, Methods Enzymol., 2, 8 (1955) and references cited therein.

14) Based on the TOC value of 1482 ppm (Table 1), the expected yield of the di(L-phenylalanine)amide of adipic acid is 69%. The difference between this and the observed 40% suggests that oligomeric metabolites may also be soluble in the buffer solution.

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