Synthesis of polyamides containing sites targeted for enzymatic cleavage

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A synthetic approach to polyamides containing the tyrosine–leucine linkage is presented. The diphenyl phosphoryl azide coupling technique was utilized to synthesize the monomer, tyrosylleucyliminohexamethyleneiminoleucyltyrosine. Solution polymerization of this monomer with adipoyl and sebacoyl chlorides resulted in polymers with intrinsic viscosities of 0.18 and 0.13 dl g^{-1}, respectively, in 90% formic acid. Interfacial polymerization yielded a fibrous crosslinked material. The latter was found to be a poly(amide–ester) by i.r. spectroscopic analysis.

(Keywords: tyrosine–leucine; polyamides; poly(amide–ester))

Introduction

The incorporation of enzyme targeted cleavage points in synthetic polymers has great potential, because it can lead to biodegradable materials based on definitive chain scission mechanisms. This approach could ensure the improved binding of an enzyme to the polymer resulting in the efficient formation of an expected enzyme–substrate complex and subsequent degradation[1]. Applications of these polymers include artificial skin substitutes, degradable sutures, drug delivery systems[2,3] and marine applications[4].

Previously, it had been demonstrated that glycine-containing nylons, such as nylon 26, nylon 266 and nylon 2626, are biodegradable, based on the release of carbon dioxide from slurry samples under attack by fungi and bacteria[5,6]. These polymers were synthesized by solution and/or interfacial polymerization methods[4–6]. In the solution method, relatively low-molecular-weight polymers were obtained. Furthermore, in the active ester method usually applied[7], there is the possibility of racemization where chiral molecules are concerned. However, the azide activation technique utilizing diphenyl phosphoryl azide (DPPA) has been used with considerable success, in both the coupling and polymerization of amino acids, to give racemization free peptides and polypeptides, respectively[7–11]. It has also been found not to affect reactive side groups, such as hydroxyl, during coupling[7]. Recently, DPPA has been utilized to make polyamides, polyureas and polyurethanes from their respective monomers[11–13].

In this paper, a synthetic approach utilizing DPPA to incorporate the dipeptide, tyrosine–leucine (Tyr–Leu) in polyamides is outlined. The polymers are subjected to degradation using enzymes such as chymotrypsin[1], thermolysin, subtilisin and aspergillopeptidase[14]. It is anticipated that these enzymes will act either on the Tyr–Leu linkage or the bond between leucine and the parent nylon structure.

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Experimental

Materials. The amino acid derivatives were obtained from Advanced ChemTech Inc., Louisville, KY, USA, and were used without further purification. DPPA was obtained from Aldrich Chemical Company and was purified by distilling under reduced pressure. Adipoyl and sebacoyl chlorides were distilled under reduced pressure while hexamethylenediamine was purified by vacuum sublimation. Triethylamine was dried over calcium hydride and distilled at atmospheric pressure. Anhydrous dimethylformamide (DMF) was obtained by drying over BaO and distilling it under reduced pressure.

Characterization. Elemental analysis (C, H, N) was done at Galbraith Laboratories Inc., Knoxville, TN, USA. The melting point of the monomer was determined using a Mel-Temp II melting point apparatus and is uncorrected. The intrinsic viscosities of the polymers were measured in 0.5 g dl^{-1} solution of 90% formic acid at 25°C using an Ubbelohde viscometer, while the i.r. spectra of the polymers (KBr pellet) were recorded on a Nicolet 60SX FTIR Spectrometer. ^1H n.m.r. spectra were obtained on an IBM AF-270 NMR Spectrometer (270 MHz), with CF_3COOD as the solvent. The molecular weight of the monomer was determined using the fast atom bombardment (f.a.b.) technique on a Kratos MS50RF High Resolution Magnetic Sector Mass Spectrometer.

Synthesis of monomer tyrosylleucyliminohexamethyleneiminoleucyltyrosine (Tyr–Leu–$(CH_2)_6$–Leu–Tyr) (I).

(i) *t*-Butyloxycarbonyl (BOC)–Tyr–Leu–OMe. To a stirred mixture of BOC–Tyr (5.63 g, 20 mmol) and Leu–OMe.HCl (4.0 g, 22 mmol) in DMF (80 ml), DPPA (6.05 g, 22 mmol) in DMF (10 ml) was added with cooling in ice. This was followed by the addition of triethylamine (TEA, 5.85 g, 42 mmol) in DMF (10 ml). The mixture was stirred with cooling in ice for 4 h and then left to react at room temperature for 20 h. The work-up of the product was achieved by diluting the reaction mixture with ethyl acetate–benzene (4:1, 800 ml) followed by washing with

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5% HCl (2 × 100 ml), saturated $NaHCO_3$ (2 × 100 ml), saturated NaCl (2 × 100 ml) and water (2 × 100 ml). The resulting organic phase was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* at 40°C. The solid obtained was dried in vacuum to give 6.70 g (82.1%) of the dipeptide BOC–Tyr–Leu–OMe. The peptide was used without further purification.

(ii) Tyr–Leu–$(CH_2)_6$–Leu–Tyr. The dipeptide (6.70 g, 16.4 mmol) was dissolved in acetone (20 ml) and reacted with NaOH (1.6 g, 40 mmol) in 20 ml water for 1 h. The acetone was removed under reduced pressure and the aqueous phase was extracted twice with diethyl ether. It was then acidified with 1N HCl to pH 3. The resulting white precipitate was immediately extracted with ethyl acetate (2 × 100 ml). The ethyl acetate phase was washed with water several times and then dried over anhydrous Na_2SO_4. The solvent was evaporated off under reduced pressure and the white solid obtained was dried in vacuum to give 6.07 g (77.0%) of the deblocked dipeptide, BOC–Tyr–Leu–OH. The ^1H n.m.r. spectrum of the sample showed no methoxy peak at around 3.72 ppm.

BOC–Tyr–Leu–OH (6.07 g, 15.4 mmol) and hexamethylenediamine (0.897 g, 7.7 mmol) in DMF (60 ml) were coupled using 4.40 g DPPA (16 mmol) and 3.62 g TEA (36 mmol) as in the case of BOC–Tyr–Leu–OMe. On work-up, 6.1 g (91.3%) of the dry crude product was obtained. This sample was reacted with trifluoroacetic acid (TFA, 20 ml) for 1 h at room temperature. After evaporating off the excess TFA, the remaining sample was triturated in ether several times. It was then recrystallized from diethyl ether–methanol (10:1). The white crystalline sample obtained was dried in vacuum to give 4.13 g (65.6%) of the TFA salt of monomer **I**, Tyr–Leu–$NH(CH_2)_6NH$–Leu–Tyr, m.p. 160°C. The m.s. (f.a.b.) gave a peak at 670 (m + 2H), for the protonated monomer without TFA anion.

Elemental analysis. Found: C, 51.89; H, 6.76; N, 8.75. Calculated for $C_{40}H_{58}N_6O_{10}F_6.2H_2O$: C, 51.50; H, 6.70; N, 9.01.

N.m.r. (CF_3COOD), δ (ppm): 0.81 (m, 12H, $(CH_3)_2$, Leu), 1.35–1.58 (m, 14H, $CHCH_2$, Leu, CH_2, diamine), 3.1–3.2 (m, 8H, CH_2NH, diamine, CH_2, Tyr), 4.5–4.6 (t, 4H, CH–N Tyr, Leu), 6.8–7.0 (d, 8H, aromatic H, Tyr). I.r. (KBr pellet): 3500–3200 cm^{-1} (O–H stretching), 3298 cm^{-1} (N–H stretching), 3080 cm^{-1} (C–H stretching, aromatic), 2930 and 2858 cm^{-1} (C–H stretching, aliphatic), 1652 cm^{-1} (amide I), 1521 cm^{-1} (amide II).

Solution polymerization.

(i) Poly(tyrosylleucyliminohexamethyleneiminoleucyltyrosyladipoyl) (PTLHLTA). The peptide TFA.Tyr–Leu–$NH(CH_2)_6NH$–Leu–Tyr.TFA (1.0 g, 1.28 mmol, monomer **I**) and 0.8 ml of freshly distilled TEA were dissolved in 5 ml of dry chloroform. To this mixture was added a solution of 0.23 g (0.19 ml, 1.28 mmol) of adipoyl chloride in 5 ml chloroform while stirring. The mixture became viscous immediately and a white solid appeared. After 5 min the product was poured into hexane (100 ml) with stirring. The resulting precipitate was filtered and washed thoroughly with water, methanol and acetone. The sample was then dried in vacuum at 60°C. A white solid (yield 0.52 g, 52.2%) was obtained with an intrinsic viscosity of 0.14 dl g^{-1} in 90% formic acid.

Elemental analysis. Found: C, 61.87; H, 8.27; N, 10.06. Calculated for $C_{42}H_{62}N_6O_8.2H_2O$: C, 61.89; H, 8.16; N, 10.31.

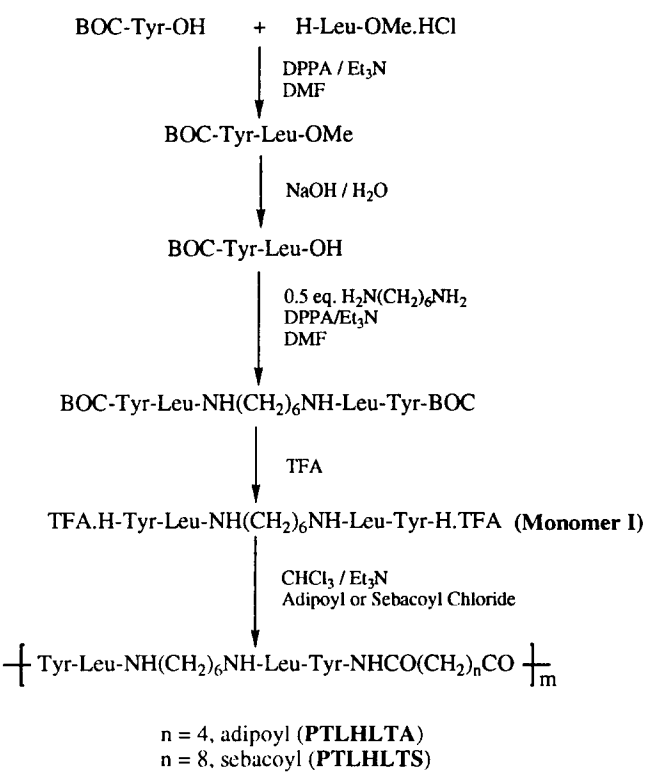
N.m.r. (CF_3COOD), δ (ppm): 0.83 (d, 12H, $(CH_3)_2$, Leu), 1.3–1.5 (m, 18H, $CHCH_2$, Leu, CH_2, diamine, adipoyl), 2.3 (m, 4H, CH_2CO, adipoyl), 2.96–3.3 (m, 8H, CH_2, Tyr, CH_2N, diamine), 4.6–4.8 (m, 4H, CH–N, Tyr, Leu), 6.8–7.0 (d, 8H, aromatic, Tyr). I.r. (KBr pellet): 3500–3200 cm^{-1} (O–H, stretching), 3317 cm^{-1} (N–H, stretching), 3084 cm^{-1} (C–H stretching, aromatic), 2933 and 2853 cm^{-1} (C–H stretching, aliphatic), 1653 cm^{-1} (amide I), 1518 cm^{-1} (amide II).

(ii) Poly(tyrosylleucyliminohexamethyleneiminoleucyltyrosylsebacoyl) (PTLHLTS). To a solution of monomer **I** (0.9 g, 1.0 mmol) and 0.6 ml of TEA in dry chloroform (15 ml) was added 0.196 g (0.82 mmol) of sebacoyl chloride dissolved in 15 ml of dry chloroform. The mixture was polymerized as in the case of adipoyl chloride. The polymer obtained (yield 0.25 g, 37%) had an intrinsic viscosity of 0.13 dl g^{-1} in 90% formic acid.

Elemental analysis. Found: C, 63.22; H, 8.48; N, 8.71. Calculated for $C_{46}H_{70}N_6O_8.2H_2O$: C, 63.42; H, 8.56; N, 9.65.

N.m.r. (CF_3COOD), δ (ppm): 0.82 (d, 12H $(CH_3)_2$, Leu), 1.2–1.5 (m, 26H, $CHCH_2$, Leu, CH_2, diamine, sebacoyl), 2.4 (m, 4H, CH_2CO, sebacoyl), 3.0–3.3 (m, 8H, CH_2, Tyr, CH_2–N, diamine), 4.6–4.8 (m, 4H, CH–N, Tyr, Leu), 6.8–7.0 (d, 8H, aromatic H, Tyr). I.r. (KBr pellet): 3500–3200 cm^{-1} (O–H stretching), 3298 cm^{-1} (N–H stretching), 3080 cm^{-1} (C–H stretching, aromatic), 2929 and 2858 cm^{-1} (C–H stretching, aliphatic), 1653 cm^{-1} (amide I), 1522 cm^{-1} (amide II).

Interfacial polymerization. An aqueous solution of monomer **I** was made by mixing 0.46 g (0.51 mmol) of the monomer with 0.082 g (2.04 mmol) NaOH in 5 ml of water. This solution was added to a solution of adipoyl chloride (0.094 g, 0.51 mmol) in chloroform (2 ml). The mixture was stirred vigorously for 5 min. The white



Scheme 1 Synthesis of Tyr–Leu–$NH(CH_2)_6NH$–Leu–Tyr polymers

fibrous material obtained was washed thoroughly with water, water–methanol (1:1), and methanol. It was then dried at 60°C in a vacuum oven to give 0.18 g (45.3%) of the product. The polymer was found to be insoluble in most organic solvents, including TFA and formic acid.

I.r. (KBr pellet): 3292 cm^{-1} (N–H stretching), 3061 cm^{-1} (C–H stretching, aromatic), 2923 and 2846 cm^{-1} (C–H stretching, aliphatic), 1754 cm^{-1} (C=O stretching, ester), 1650 cm^{-1} (amide I), 1523 cm^{-1} (amide II). The same procedure as described for adipoyl chloride polymerization was followed with sebacoyl chloride, and similar results were obtained.

Results and discussion

Scheme 1 gives the general outline of synthesizing adipoyl or sebacoyl polymers containing the Tyr–Leu–$(CH_2)_6$–Leu–Tyr unit.

Solution polymerization of monomer **I** was achieved by reacting a chloroform solution of the monomer in TEA with chloroform solutions of either adipoyl or sebacoyl chlorides to give the corresponding polymers[15]. The adipoyl polymer (PTLHLTA, yield 0.52 g, 52.2%) had an intrinsic viscosity of 0.14 dl g^{-1} while the sebacoyl (PTLHLTS, yield 0.25 g, 37%) had an intrinsic viscosity of 0.13 dl g^{-1} in 90% formic acid. *Figure 1* gives the structures of the two polymers.

The interfacial polymerization of monomer **I** was carried out by reacting the sodium hydroxide solution of the monomer with chloroform solutions of either adipoyl or sebacoyl chloride[4]. A crosslinked poly(amide–ester) was obtained in each case, as indicated by the insolubility of the polymer in most organic solvents as well as TFA, *m*-cresol and formic acid[16]. The chances of

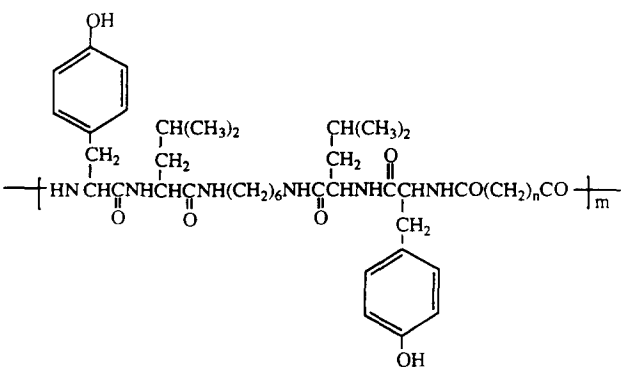
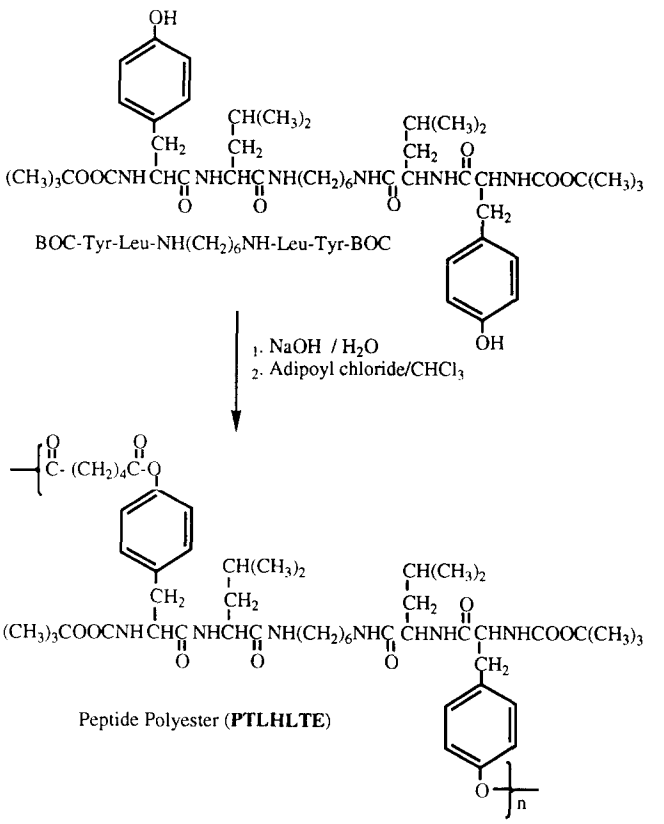


Figure 1 Structure of poly(tyrosylleucyliminohexamethyleneimino-leucyltyrosyladipoyl) (PTLHLTA), $n=4$, and poly(tyrosylleucylimino-hexamethyleneiminoleucyltyrosylsebacoyl) (PTLHLTS), $n=8$



Scheme 2 Synthesis of peptide polyester by interfacial polymerization

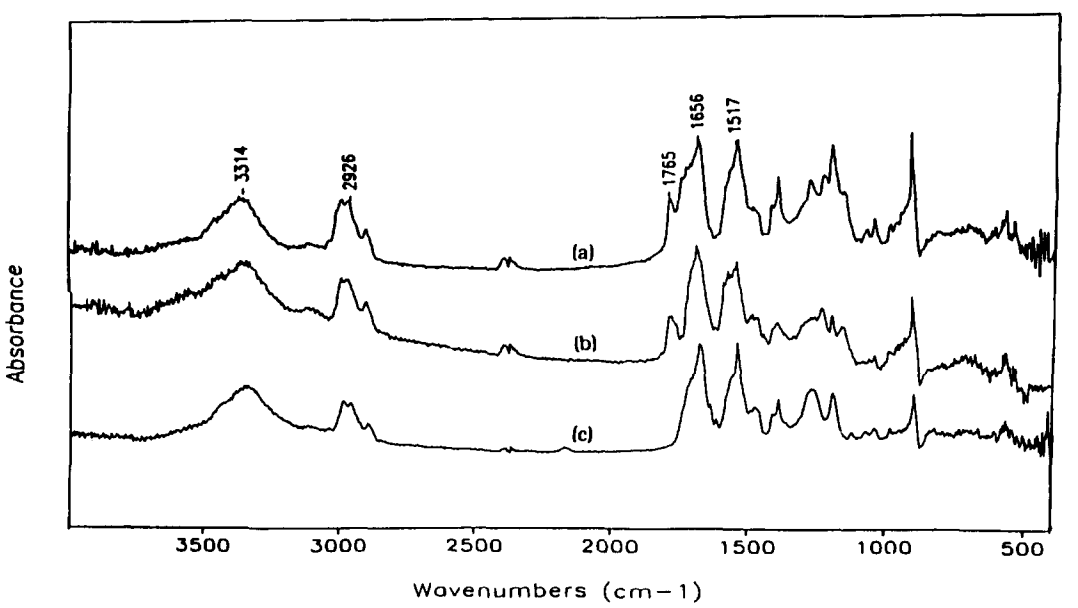


Figure 2 Infra-red spectra of the polymers: (a) poly(amide–ester), crosslinked; (b) peptide polyester; (c) polyamide, PTLHLTA

an intramolecular type reaction of monomer **I** to form a ring system are remote, because the long chain of 30 atoms decreases the statistical probability of the end groups cyclizing on reaction with a diacyl chloride[17]. The formation of crosslinked polymer can be attributed to the tetrafunctionality of the monomer, i.e. due to the reaction of both the amino and hydroxyl groups in the tyrosine unit with acid chloride in alkaline medium. In order to confirm the reaction due to the hydroxyl group in the tyrosine unit, interfacial polymerization of an alkaline solution of amino protected monomer **I** and a chloroform solution of adipoyl chloride was carried out (*Scheme 2*). A peptide polyester, poly(tyrosylleucyl-iminohexamethyleneimino-leucyltyrosyladipoyl) ester (PTLHLTE), was obtained. It had an intrinsic viscosity of 0.18 dl g^{-1} in 90% formic acid. The i.r. spectrum of PTLHLTE was compared with that of the crosslinked poly(amide–ester). The two spectra had peaks at 1765 cm^{-1} (ester), 1656 cm^{-1} (amide I) and 1517 cm^{-1} (amide II)[11,18]. The amide peaks in PTLHLTE can be attributed to the peptide bonds originally present in the monomer. Although the i.r. spectrum of the polymer made by solution polymerization of monomer **I** (PTLHLTA) did not show the ester peak at 1765 cm^{-1} (*Figure 2*), under the reaction conditions (excess of TEA) a partial reaction via the hydroxyl groups of tyrosine cannot be precluded. The presence of a minor amount of ester bonds would not be detectable at 1765·cm^{-1}.

Conclusions

Initial studies indicate that the DPPA technique has been successful in synthesizing the monomer containing the tyrosine–leucine peptide linkage, a potential target for enzymatic degradation. The peptide monomer has been utilized to synthesize sequential polymers by both solution and interfacial polymerization methods. Modifications to obtain higher molecular weight polymers are underway.

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