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Synthesis and structural study of a new biodegradable copolymer of nylon-11 and L-alanine

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The alternating copolyamide, poly(L-alanyl-11-aminoundecanoic acid), has been synthesized by the active ester method, and subsequently characterized. The structure and morphology of lamellar crystals have been investigated by using transmission electron microscopy, selected-area electron diffraction and X-ray diffraction. Additional data have been obtained from uniaxially oriented fibres. The deduced unit cell parameters ($a = 4.79$, $b = 10.35$, $c = 29.8$ Å, and $\alpha = \beta = \gamma = 90°$) and the $C222_1$ space group symmetry indicate a layered structure which is related to that found in poly(L-alanine). A folded conformation is also deduced for the polymethylene segment. Results obtained from enzymatic degradation are also reported.

(Keywords: alanine; biodegradation; nylon-11)

INTRODUCTION

Synthetic poly(α-amino acid)s and their copolymers have been widely studied as a potential family of biodegradable and compatible polymers[1–4], where the degradation is attributed to the cleavage of amide linkages by proteolytic enzymes. Furthermore, it has been demonstrated that their rate of *in vivo* degradation can be controlled by varying the hydrophilicity of the side-chain groups[5]. Nevertheless, practical applications of these copoly(amino acid)s are still limited, partly because of difficulties in their preparation and certain physical properties such as low thermal stability and high melting temperature. In this sense, the utilization of α-amino-acid-containing copolyamides is of interest, since (a) the materials may be still biodegradable due to the incorporation of α-amino acids, as has been shown for some glycine copolyamides[6,7] and nylon-6 derivatives[8], and (b) physical properties may be greatly modified according to the α-amino acid counterparts. Additional interest derives from the fact that the structure of these copolyamides may be different from the conventional α- and γ-forms of nylons, depending on the structural preferences of the α-amino acid. Therefore, certain alternating glycine copolyamides assume a polyglycine II structure[9], which is characterized by a three-dimensional network of hydrogen bonds which tightens the whole structure[10–14].

In this present article, we report the synthesis and characterization of a new alternating copolyamide consisting of L-alanine and 11-aminoundecanoic acid. At the present time, no structural data have been reported for nylons which have incorporated optically active α-amino acids. In this sense, we have selected alanine as the most simple L-amino acid, while the 11-aminoundecanoic comonomer has been chosen in order to favour the melt processability of the polymer, as expected from the resulting high methylene/amide ratio.

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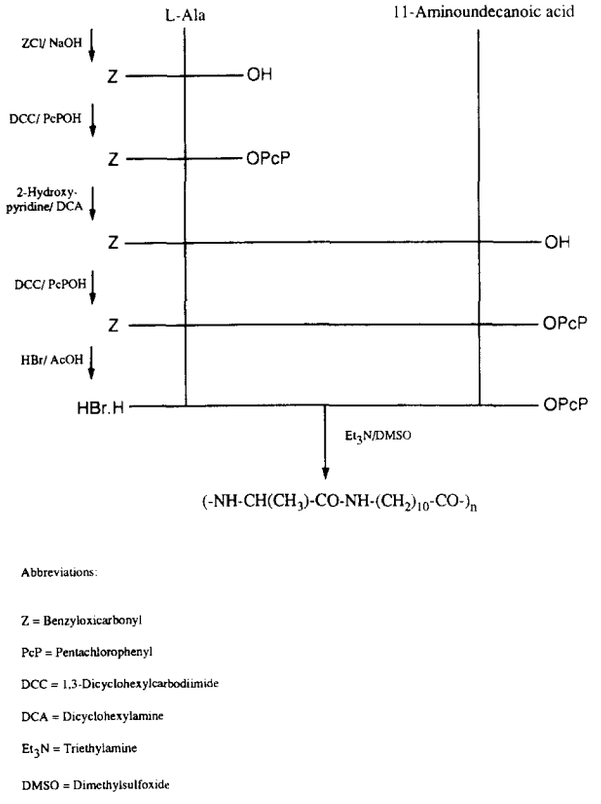
EXPERIMENTAL

Synthesis

The monomer was synthesized in solution by applying the widely known methodology developed in peptide synthesis[15] and outlined in *Scheme 1*. The *N*-carbobenzyloxy-L-alanine and the *N*-carbobenzyloxy-L-alanine pentachlorophenyl ester were prepared according to published procedures[16,17]. Other required reagents were obtained commercially and used without further purification.

Coupling reaction. A mixture of 100 g (0.21 mol) of *N*-carbobenzyloxy-L-alanine pentachlorophenyl ester, 0.1 g of 2-hydroxypyridine, 42 g (0.21 mol) of 11-aminoundecanoic acid, 42 ml (0.21 mol) of dicyclohexylamine and 400 ml of CH_2Cl_2 was stirred at room temperature for 48 h, filtered and then concentrated *in vacuo*. The solid residue was dissolved in 320 ml of ethyl acetate, and 130 ml of 2 M HCl were added to precipitate the hydrochloride salt of dicyclohexylamine. After filtration, the solution was extracted with ethyl acetate. The organic layer was concentrated *in vacuo* and extracted with a 5% solution of sodium bicarbonate. After acidification with 1 M HCl, a white powder was recovered. The solid product was redissolved in ethyl acetate and then crystallized by the slow addition of petroleum ether; yield 49%, m.p. 67°C. Elemental analysis: calculated for $C_{22}H_{34}N_2O_5$, C 65.02, H 8.37, N 6.90%; found, C 65.21, H 8.58, N 6.70%.



Scheme 1

Preparation of the activated pentachlorophenol ester derivative. To a stirred ice-cooled solution of 40 g (0.1 mol) of *N*-carbobenzyloxy-L-alanyl-11-aminoundecanoic acid in 100 ml of CH_2Cl_2, a solution of 23 g of dicyclohexylcarbodiimide (DCC) (0.11 mol) and pentachlorophenol (30 g, 0.11 mol) in 100 ml of CH_2Cl_2 was slowly added. The mixture was stirred at room temperature overnight, followed by the addition of 8 ml of glacial acetic acid to decompose the excess DCC. The dicyclohexylurea was filtered off and the solution evaporated under reduced pressure. The resulting solid was recrystallized from isopropanol; yield 55%, m.p. 109°C. Elemental analysis: calculated for $C_{28}H_{33}Cl_5N_2O_5$, C 51.34, H 5.04, N 4.28%; found, C 51.49, H 5.25, N 4.15%.

Removal of the protecting group. The protected amino acid (33 g, 0.05 mol) was dissolved in 400 ml of glacial acetic acid containing 15% HBr. The initial vigorous evolution of carbon dioxide ceased after ca. 30 min. After standing for 30 min at room temperature, the hydrobromide salt was precipitated with ethyl ether. The remaining solid was extensively washed with ether and finally dried in a vacuum desiccator; yield 70%. Elemental analysis: calculated for $C_{20}H_{28}BrCl_5N_2O_3$, C 39.91, H 4.65, N 4.65%; found, C 39.57, H 4.75, N 4.37%.

Polymerization. The monomer (10 g, 16 mmol) was suspended in 10 ml of dimethyl sulfoxide (DMSO) and 5 ml (36 mmol) of Et_3N was added slowly while the system was agitated. The solid dissolved immediately and the solution turned gradually darker in colour and more and more viscous in consistency. The reaction mixture was then allowed to stand for 5 days after agitation for ca. 20 min. After precipitation with diethyl ether, the solid was triturated and filtered. The recovered white powder was extensively washed with ethanol, water, ethanol and ether (yield 90%).

Thermal post-polycondensation was achieved by heating the polymer samples at 200°C for 1 h under a nitrogen atmosphere. I.r. (KBr) cm^{-1}: 3280 (amide A), 3062 (amide B), 2916 and 2844 (C–H), 1628 (amide I), 1538 (amide II). ^1H n.m.r. (trifluoroacetic acid (TFA-d) δ: 4.72 (1H, CH), 3.39 (2H, $-NHCH_2-$), 2.58 (2H, $-CH_2CO-$), 1.71 (4H, NH $CH_2CH_2(CH_2)_6CH_2CH_2CO-$), 1.56 (3H, CH_3), 1.32 (12H, –NH $CH_2CH_2(CH_2)_6CH_2CH_2CO-$). ^{13}C n.m.r. (TFA-d) δ: 182.39 (CO, aminoundecanoic), 176.15 (CO, alanine), 53.47 (CH), 43.37 ($-NHCH_2-$), 36.75 ($-CH_2CO-$), 18.34 (CH_3). Elemental analysis: calculated for $C_{14}H_{26}N_2O_2$, C 66.14, H 10.24, N 11.02%; found, C 64.02, H 9.73, N 10.57%.

Characterization

The intrinsic viscosity of the polymer was determined with a Cannon-Ubbelohde microviscometer at a temperature of 25.0 ± 0.1°C; dichloroacetic acid was used as a solvent. Infra-red (i.r.) spectra were obtained on KBr pellets using a Perkin–Elmer 783 spectrophotometer in the 4000–500 cm^{-1} range. Proton and carbon nuclear magnetic resonance (^1H and ^{13}C n.m.r.) spectra were obtained in deuterated trifluoroacetic acid using a Bruker AMX-300 spectrometer. Chemical shifts are reported in parts per million (δ) downfield from internal tetramethylsilane (TMS). The thermal behaviour of the polymer was investigated by differential scanning calorimetry (d.s.c.) using a Perkin-Elmer DSC-4 machine equipped with a TADS data station, at a heating rate of 10°C min^{-1} in a nitrogen atmosphere. Temperature and heat of fusion were calibrated with an indium standard (T_m = 429.75 K, ΔH_f = 3.267 kJ mol^{-1}). The density of the powder samples was measured at 25°C by the flotation method, using a mixture of benzene and carbon tetrachloride.

Structural methods

Crystallization experiments were carried out isothermally using dilute solutions in 1,4-butanediol. The crystals were recovered from the mother solutions by centrifugation and were repeatedly washed with n-butanol.

For electron microscopy the crystals were deposited on carbon-coated grids which were then shadowed with Pt/carbon at an angle of 15°. A Philips EM-301 electron microscope operating at either 80 or 100 kV for bright field and electron diffraction, respectively, was used throughout this work. Electron diffraction patterns were internally calibrated with gold (d_{111} = 2.35 Å). Polymer decoration was achieved by evaporating polyethylene over the surface of single crystals, as described by Wittmann and Lotz[18]. X-ray diagrams were recorded under vacuum at room temperature. Calcite (d_B = 3.035 Å) was used for calibration. A modified Statton camera (W. R. Warhus, Wilmington, DE) with nickel-filtered copper radiation of wavelength 1.542 Å was used for these experiments. Patterns were recorded from

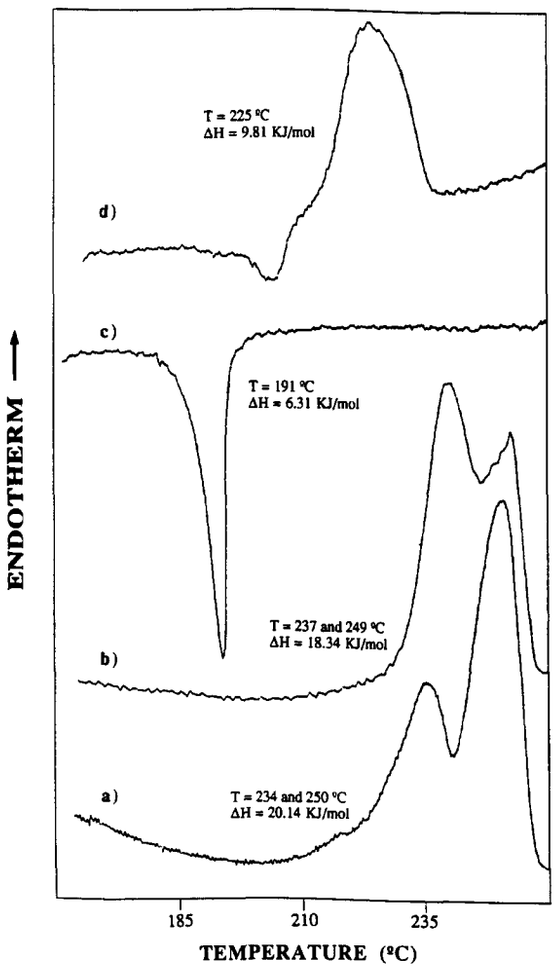


Figure 1 D.s.c. traces of poly(L-alanyl-11-aminoundecanoic acid): (a) polymer sample direct from solution polymerization; (b) thermal post-polymerized sample; (c) cooling curve of (b); (d) heating curve of the sample crystallized in (c). In all cases, the heating/cooling rate was 10°C min^{-1}

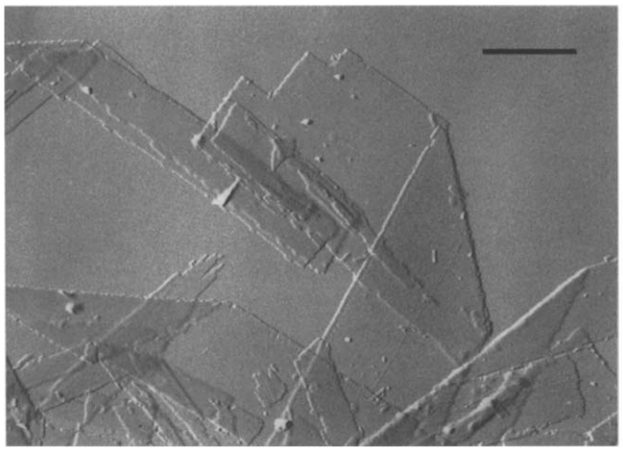


Figure 2 Transmission electron micrographs of poly(L-alanyl-11-aminoundecanoic acid) lamellar crystals. The ribbon-like lamellae were obtained from butanediol solutions at 150°C. Note the regular and orthogonal edges of the crystals (scale bar represents 1 μm)

either polymer powders, fibres or from mats of single crystals which were prepared by slow filtration of a crystal suspension on glass filter.

Enzymatic hydrolysis

Polymer degradation was carried out by using papain as a proteolytic enzyme in a 0.05 M phosphate buffer (pH = 6.0). Papain (30 000 USP-U mg^{-1}, No. 7144) was purchased from Merck, and was used without further recrystallization. The enzyme was activated in the buffered solution with 34 mM L-cysteine and 38 mM ethylenediaminetetraacetic acid (EDTA) as proposed by Arnon[19]. Sodium azide (0.02% (wt/vol)) was used to prevent microbial growth. The stability of the enzyme under the conditions of the experiment was controlled by the use of a high-molecular-weight substrate such as casein. The activity of the papain was determined by measuring the absorbancy of the solution at 280 nm.

Polymer powder samples (initial weight, 50 mg) were placed in small bottles containing 10 ml of the enzymatic medium. The reaction solution was incubated at 37°C, with shaking, from 1 to 72 h. The enzyme was not regenerated, since the measured activity loss was only 15%. After the reaction, the enzyme was inactivated by adding 1 ml of 1 M HCl and the polymer weight loss was measured after centrifugation and drying to constant weight *in vacuo*. Hydrolytic degradation was also monitored in the phosphate buffer solution during equivalent exposure times.

RESULTS AND DISCUSSION

Polymer characterization

The intrinsic viscosity of the solution-polymerized sample was low, indicating that polycondensation has not progressed as far as was expected. Different reaction conditions were tested in order to increase the degree of polymerization, but no significant improvement could be achieved. A limiting viscosity number of ca. 0.32 dl g^{-1} was found for the resulting polymer. However, the molecular weight was clearly improved by thermal post-polycondensation, giving samples of adequate intrinsic viscosity (0.52 dl g^{-1}) for film or fibre processing. A molecular weight of ca. 8500 could be estimated if the viscosimetric equation for nylon-66 is applied to the calculation[20]. Thermal treatment was carried out in sufficiently mild conditions to avoid side reactions and degradation, as was demonstrated from the i.r. and n.m.r. spectra which were in agreement with the expected polymer composition.

Double melting peaks are seen for either solution-polymerized or thermal post-polycondensate samples, a common observation in thermal studies of nylons[21]. Although both the total heat of fusion and the melting temperatures are similar for the two samples, the main endothermic peaks are seen to change (*Figures 1a* and *1b*). Thus the solution-synthesized sample appears to be enriched with the 'best' crystals, which have a high melting temperature. A crystallinity of ca. 39% can be calculated for both samples, using as a first approximation the reported[22] group contribution to the heat of fusion of the amide, methyl and methylene groups. A single exothermic peak was observed in the cooling run of the melted polymer samples. However, crystallization appears to be hindered, as deduced from the low heat of crystallization (only 30% of the fusion heat), indicating that presumably some decomposition takes place after fusion. Furthermore, a slight brown coloration

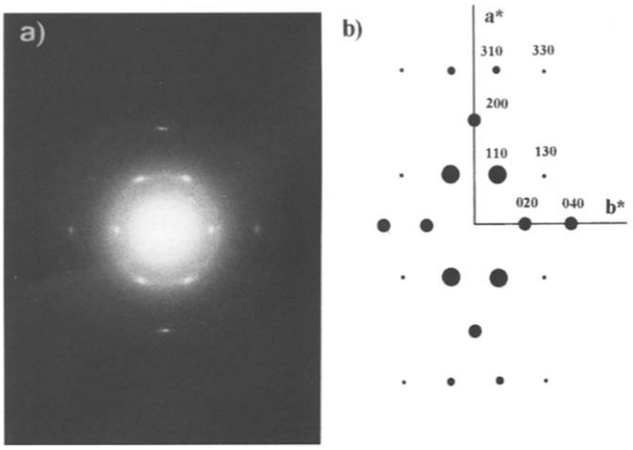


Figure 3 (a) Electron diffraction pattern of single crystals of poly(L-alanyl-11-aminoundecanoic acid) obtained from 1,4-butanediol. (b) Schematic representation of the reflections contained in the electron diffraction pattern

was detected in the melt-crystallized samples and the heating traces of the latter 2nd scan (*Figure 1d*) do not well reproduce the original melting peaks. The single endothermic peak was moved to lower temperatures and its enthalpy decreased greatly (around 50%). This fusion peak was always preceded by a small exotherm, which was indicative of cold crystallization.

Electron microscopy

Crystallization from 0.1% (wt/vol) solutions in 1,4-butanediol at 150°C yielded long single crystals, as depicted in *Figure 2*. The lamellae frequently appeared to be folded over themselves due to their ribbon-like morphology. In spite of the highly variable length and width dimensions of the crystals (from 2 to 8 μm and 0.7 to 2 μm, respectively), a rather homogeneous thickness ca. 65 Å) has been estimated from their shadows in the micrographs. It is worth noting the well-defined edges of the crystals which form angles of 90°. This orthorhombic morphology is also evident when the front edges appear steeped, as also shown in *Figure 2*. The serrated edges displayed by some crystals in the micrographs probably correspond to breakage occurring in the sample preparation process. Fine striations which run

Table 1 Measured and calculated diffraction spacings d_B (Å) for different samples of poly(L-alanyl-11-aminoundecanoic acid)[a]

						X-ray diffraction					
		Electron microscopy									
Index[b]	Calculated	(single crystal)		Powder		Mat			Fibre		
Lamellar thickness	65					65[c]	vs	M			
2nd order	32.5					32[c]	w	M			
3rd order	21.7					21.5	w	M			
4th order	16.2					16.1	s	M			
5th order	13.0					13.1	m	M			
6th order	10.8					10.8	w	M			
7th order	9.3					9.3	w	M			
002	14.9			14.9	s				14.9	vs	M
004	7.45								7.45	vw	M
020	5.17	5.17	s	5.10	s	5.17	m	E	5.17	w	E
022	4.88					4.85	m				
023	4.59					4.60	w				
110	4.35	4.34	vs	4.35	vs	4.35	vs	E	4.34	vs	E
112, 024	4.18, 4.24					4.22	vs				
113	3.98			4.00	m	4.00	s				
115, 026	3.51, 3.58			3.60	w	3.55	m				
116, 027	3.27, 3.28					3.28	w				
117, 028	3.04, 3.02			3.04	vw	3.02	w				
118, 029	2.83, 2.79			2.81	vw	2.78	vw				
130	2.80	2.80	w								
040	2.59	2.58	s	2.56	w	2.55	m				
200	2.40	2.40	s	2.41	w	2.41	m				
310	1.58	1.59	m								
330	1.45	1.46	w								

[a] Abbreviations denote intensities or orientations: vs, very strong; s, strong; m, medium; w, weak; vw, very weak; M, meridional; E, equatorial
[b] On the basis of an orthorhombic unit cell: $a = 4.79$, $b = 10.35$, $c = 29.8$ Å
[c] Observed only in low-angle X-ray patterns

parallel to their long sides can also be observed and these appear as a characteristic of the wider crystals.

The electron diffraction patterns of isolated crystals exhibit a mm symmetry until almost 1.4 Å resolution and confirm the single-crystal character of the ribbons (*Figure 3*). The *hk*0 diagram can be indexed as a centred rectangular unit cell with parameters $a = 4.79$ and $b = 10.35$ Å (*Table 1*), which is very close to the reported[23] data for the β-structure of poly(L-alanine) (orthorhombic unit cell with $a = 4.73$ and $b = 10.54$ Å). Thus a sheet structure may be deduced, with the *a*-parameter corresponding to the distance between the hydrogen-bonded chains. It is worth noting that an orthorhombic packing is closer to the sheet structure of polypeptides than the layered structure of nylons where triclinic or monoclinic unit cells are found. Furthermore, the intersheet distance (5.17 Å, as deduced from the 020 reflection) is the expected one for methyl substituents in the molecular chain (5.27 Å in poly(L-alanine)).

The meridional spot at 2.40 Å (200 reflection) appears oriented along the direction of maximum crystal elongation, according to the fact that the direction of the hydrogen bonds corresponds to the preferred growing direction. Furthermore, the diffraction pattern indicates that the molecular chains are perpendicular to the basal crystal faces and are folded as a consequence of their molecular weight and the reduced lamellar thickness. (Approximately 6 folds are required to accommodate the molecular chains within the height of the lamella.) In order to ascertain the folding habit in the crystal surface we have made use of the polyethylene decoration technique developed by Wittmann and Lotz[18]. The decoration shown in *Figure 4* presents regular striations perpendicular to the long faces of the crystals. By analogy with polyethylene, this can be interpreted as an indication that the molecular chains are folded parallel to the long axis of the crystals and consequently along the hydrogen-bonded sheets. Thus, the crystals consist of an arrangement of antiparallel-chain sheets which is a consequence of the fact that the hydrogen bonding in poly(ω-amino acids) (polypeptides, as well as nylons) is improved when the molecular chains have an antiparallel disposition. This arrangement seems to be in contradiction with the halving of the *a*-axis dimension from its expected value (2×4.79 Å). This problem was also observed in poly(L-alanine)[23] and a similar explanation can be given in this case. Thus, the structure is based on a statistical packing in which consecutive antiparallel-chain sheets are stacked with a random displacement of $\pm 1/2 \times 4.79$ Å in the *a*-direction. This packing is equivalent to one in which each chain site is occupied by one half of an 'up-chain' and one half of a 'down-chain'. This statistical arrangement would be likely since the intersheet interaction should be similar for the two displacements, in the same way as it occurs in poly(L-alanine). Furthermore, from the systematic absences observed in the electron diffraction pattern (*h*00, h = odd; 0*k*0, k = odd; *hk*0, $h + k$ = odd), a high symmetry can be deduced for the crystal structure. In this sense, a statistical distribution of up- and down-chains gives additional symmetry elements which fit well with the deduced space group (see below).

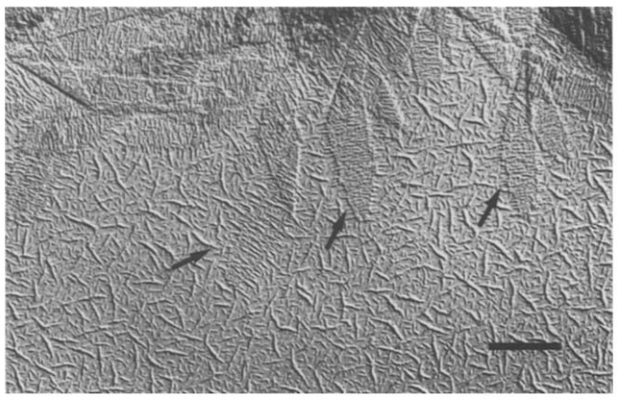


Figure 4 Electron micrograph of poly(L-alanyl-11-aminoundecanoic acid) crystals grown from 1,4-butanediol. The crystals are decorated with polyethylene and shadowed with Pt/C at an angle of 15°. Note that the decorating rods are frequently orientated perpendicular to the long edges (scale bar represents 1 μm)

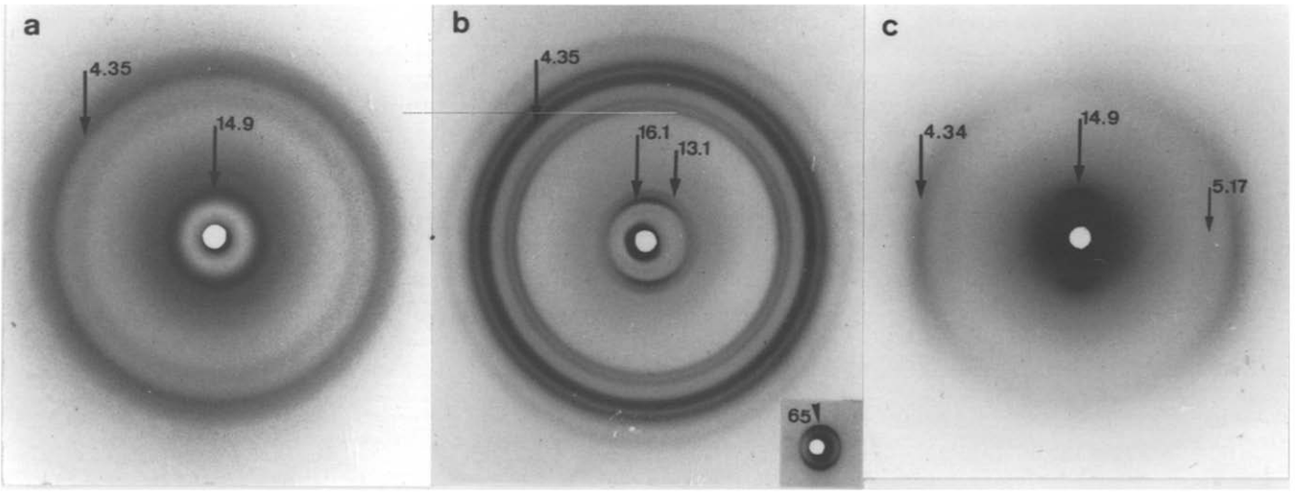


Figure 5 (a) Powder diffraction pattern of a sample recovered from polymerization. (b) Wide-angle diffraction pattern from a mat of sedimented crystals. The diffuse ring at ca. 14.9 Å in the powder pattern is replaced by two meridional reflections which are related to the lamellar orders. A meridional reflection (65 Å) corresponding to the lamellar thickness is present in the low-angle pattern (inset). (c) Diffraction pattern of an oriented fibre obtained directly from the melt

X-ray diffraction

A unique structure may be inferred from analysis of the diffraction patterns of different samples (*Figure 5*), i.e. (a) powder recovered directly from the synthesis medium, (b) a mat of sedimented crystals obtained from a 1,4-butanediol solution at 150°C and (c) fibres prepared from the melt. The measured spacings are similar within experimental error and give an average unit cell with the following parameters: $a = 4.79$, $b = 10.35$, $c = 29.8$ Å, and $\alpha = \beta = \gamma = 90°$ (*Table 1*), which agrees with the values derived from the electron diffraction patterns. The calculated density for the proposed dimensions of a centred unit cell is 1.083 g ml^{-1}, which is in good agreement with the value of 1.078 g ml^{-1} which has been experimentally observed. Some interesting features deduced from the diffraction patterns are worth discussing in more detail:

(a) The powder diffraction pattern is very diffuse due to the low crystallinity of the sample recovered directly from the polymerization medium. However, it is interesting to note both the presence of a strong low-angle reflection (indexed as 002) related to the chain repeat unit and the absence of reflections associated with lamellar spacings. On the contrary, all of the low-angle reflections observed in the diffraction pattern of the mat can be indexed as being of lamellar orders.

(b) Although the hindered crystallization from the melt gives a poor fibre diffraction pattern, an orthorhombic unit cell can be deduced from the meridional orientation of the 002 reflection.

(c) The experimental value of the unit repeat length (14.9 Å) is very different to that expected (ca. 18.5 Å) for an extended conformation. This shortening arises from a folded conformation of the aminoundecanoic unit, which takes place in order to increase the attractive van der Waals interactions between the neighbouring layers. Thus, the methyl group of the alanine residues causes a separation of 5.17 Å between successive sheets, which is very different to the optimum 3.8 Å separation usually found in nylons with an extended conformation. Furthermore, an unrealistic density of 0.87 g ml^{-1} can be predicted for an extended-chain structure. The folding of the aminoundecanoic residue appears as the only way to obtain a more compact structure without the inclusion of solvent molecules. In fact, our previous results on certain oligomers[24,25] and polymers[26] demonstrate that an extended conformation for the polymethylene segments is not always preferred.

(d) The most intense reflection corresponds to the 110 chain packing spacing and appears with an equatorial orientation in both fibre and sedimented crystal patterns. An equatorial orientation can also be detected for the 020 medium-intensity reflection. Both observations are full in agreement with the electron diffraction patterns.

(e) A lamellar thickness of 65 Å can be deduced with precision from the meridional reflections observed in the low- and wide-angle diffraction patterns of sedimented crystals. This thickness is similar to the value measured by electron microscopy and corresponds to only a little more than four repeat units (ca. 60 Å).

Table 2 Degradation of poly(L-alanyl-11-aminoundecanoic acid) showing weights of the samples after 72 h of incubation

Incubation time (h)	Weight remaining after enzymatic treatment (mg(%))	Weight remaining after hydrolytic degradation (mg (%))
0	50 (100)	50 (100)
9	45.5 (91.0)	49.5 (99.0)
13	45.7 (91.4)	48.7 (97.4)
18	42.7 (85.4)	49.1 (98.2)
20	32.0 (64.0)	49.6 (99.2)
24	16.5 (32.3)	49.2 (98.4)
40	15.0 (30.0)	49.7 (99.4)
48	9.2 (18.4)	48.9 (97.8)
72	6.1 (12.2)	49.4 (98.8)

(f) A high number of lamellar orders (up to seven) have been detected in the crystalline samples, indicating that the lamellar width is rather constant. The fourth and fifth lamellar orders are very intense in the patterns obtained from the mats, as can be predicted from the fact that the molecular repeat unit should fall between these diffraction orders. As found for other polyamides studied by us[11,27], the diffraction pattern corresponds to a stacking of lamellae in crystallographic register. In contrast, a pattern dominated by subsidiary maxima, which suggests that the individual lamellar crystals diffract independently, has been found by Keller and coworkers for nylon-66[28,29].

From the reported results, the polymer seems to consist of a statistical arrangement of antiparallel-chain sheets. These sheets are parallel to the *ac*-plane, with the hydrogen bonds being established along the *a*-direction. Chains in one sheet are centred on the unit-cell origin and those from neighbouring sheets pass through the centre of the unit cell. A random displacement of $\pm 0.5\,a$ in the hydrogen bond direction is responsible for the statistical arrangement. The molecular symmetry is characterized by a two fold screw axis, as deduced from the $00l$ (l = odd) absences detected in the diffraction patterns. An evaluation of the torsional angles of the aminoundecanoic moiety is not possible with the available data, but a folded conformation, however, is conclusively demonstrated. A $C222_1$ space group is the expected one when corresponding molecules of all of the sheets are at the same height in the cell. The systematic absences in the X-ray and electron diffraction patterns are fully compatible with this symmetry and allow us to discard a $P2_12_12_1$ symmetry, which would be derived when the molecules of neighbouring sheets have a *c*-axis translation. On the other hand, four repeat units have to be included in the cell according to the experimental density. This value is well explained with a $C222_1$ space group (multiplicity equal to 8) when taking into account the half-occupancy of the up- and down-chains.

Enzymatic hydrolysis

The degradability of the copolyamide was investigated by using powdered samples suspended in phosphate buffer. In a series of experiments, the susceptibility of this copolyamide to enzymatic attack was verified with papain. The progressive weight loss of the samples as a

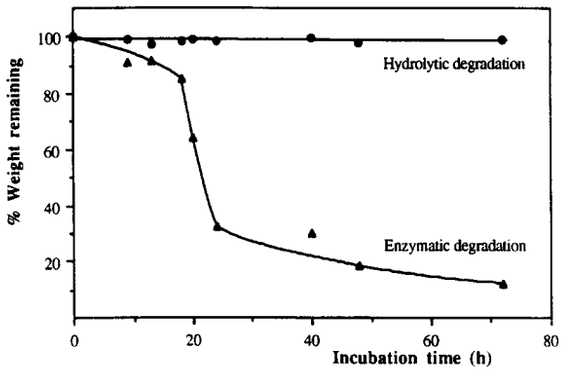


Figure 6 Weight changes in poly(L-alanyl-11-aminoundecanoic acid) samples during *in vitro* hydrolytic degradation in 0.05 M phosphate buffer (●) and enzymatic degradation with papain in 0.05 M phosphate buffer (▲)

function of the time of treatment appears to demonstrate the enzymatic biodegradation of this copolyamide.

In another series of experiments performed in parallel, papain was not added to the suspension, and thus only the action of the buffer on the polymer was studied. In this case, the weight of the recovered samples remained essentially unchanged during the selected time period, which was limited to 72 h for the sake of experimental convenience.

The weight data corresponding to both series of experiments are listed in *Table 2*, while the weight-change profiles of the poly(L-alanyl-11-aminoundecanoic acid) degradation are presented in *Figure 6*. This figure shows that the fast enzymatic biodegradation with papain causes a weight loss of 87.8% of the sample after 72 h of treatment. On the other hand, the straight line obtained from the experiment carried out without papain clearly indicates that no hydrolytic degradation has occurred during the same time period. However, owing to the short period of time in which the hydrolytic degradation has been followed in this work, it is not possible to confirm conclusively that this copolyamide does not undergo hydrolytic degradation over extended time periods.

CONCLUSIONS

The following conclusions can be drawn from the result presented here:

1. Low-molecular-weight samples are obtained from solution polymerization of active esters. However, after thermal post-polycondensation the molecular weights of the copolymers of L-alanine and 11-aminoundecanoic acid increase sufficiently to give film or fibre-forming samples.
2. Poly(L-alanyl-11-aminoundecanoic acid) crystallizes in the form of chain-folded lamellae with a thickness of ca. 65 Å. These crystals can be stacked in crystallographic register, giving up to more than seven orders in the corresponding diffraction patterns.
3. We have determined unambiguously from the X-ray and electron diffraction patterns that the crystal structure of poly(L-alanyl-11-aminoundecanoic acid) is orthorhombic, with the following unit cell dimensions: $a = 4.79$, $b = 10.35$, $c = 29.8$ Å.
4. The experimental evidence indicates a sheet structure with a single hydrogen-bond direction. Successive sheets are statistically arranged to give a $C222_1$ space group.
5. The shortening of the chain repeat length (ca. 3.5 Å from an extended conformation) and the experimental density indicate a folded conformation for the aminoundecanoic residues.
6. Enzymatic incubation with papain demonstrates the biodegradability of the poly(L-alanyl-11-aminoundecanoic acid).

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