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Structural Study on Poly(β-L-aspartate)s with Short Alkyl Side Chains: Helical and Extended Crystal Forms

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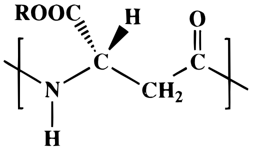
ABSTRACT: The conformation of three poly(α-alkyl β-L-aspartate)s, abbreviated PAALA-*n*, with the alkyl group being methyl, ethyl, and propyl (n = 1, 2, and 3), was examined by X-ray diffraction, polarized infrared spectroscopy, and molecular mechanics calculations. The crystal structure was modeled and refined against diffraction data with the linked-atom least-squares methodology (LALS). Two crystal forms reminiscent of the well-known α-helix and pleated β-sheet structures typical of polypeptides were characterized for the methyl derivative. The helical form is a pseudohexagonal lattice of right-handed 17/4 helices stabilized by intramolecular hydrogen bonds. The β form is made of chains in nearly extended conformation and hydrogen bonded to each other to form sheets which stack together in a metrically orthorhombic crystal lattice. In addition to these two forms, a second folded form made of right-handed 4/1 helices arranged in a tetragonal structure was characterized for the ethyl derivative. Poly(α-propyl β-L-aspartate) was found to display a crystal dimorphism entailing the hexagonal and tetragonal helical forms whereas no signs of extended conformation were detected for this polymer. In this case, the hexagonal form was found to be composed of right-handed 13/4 helices, which is the conformation usually observed in poly(β-L-aspartate)s with linear alkyl side chains of medium and larger sizes. The structural behavior exhibited by the three compounds under study is consistent with the general pattern described for PAALA-*n* with *n* ranging from 4 to 22 which have been previously studied by us in full detail.

Introduction

In 1984 we reported on a nylon 3 derivative which is able to adopt helical structures with features similar to those of the α-helix of polypeptides.[1] The polymer in question is poly(α-isobutyl β-L-aspartate), a poly(β-amide) bearing an isobutoxycarbonyl group stereoregularly attached to the β-carbon of the repeating unit. Since then, a good deal of research has been carried out on a variety of linear alkyl esters of poly(β-L-aspartic acid) covering multiple aspects related to the synthesis, crystal structure, and properties.[2−4] The acronym PAALA-*n* is used to term this family of compounds, with *n* standing for the number of carbon atoms contained in the alkyl side chain. The general chemical formula of PAALA-*n* is



The most frequent helical arrangement found in poly(β-L-aspartate)s is the right-handed 13/4 helix with intramolecular hydrogen bonds set between the *i* and *i* + 3 residues. This conformation was first described for the isobutyl ester crystallized in the so-called hexagonal form, which is actually a monoclinic lattice of up-and-down chains in the $P2_1$ space group.[1,5]. Further studies showed the 13/4 helix to be also the conformation shared by PAALA-*n* with linear alkyl side chains containing up to 22 carbon atoms.[6,7] The mode of packing and degree of ordering achieved by these polymers in the solid state have been found to be largely dependent on the length of the alkyl side group. Whereas the butyl derivative crystallizes in a pseudohexagonal lattice similar to that observed for the isobutyl isomer, members with $n \geq 12$ form bidimensional structures with the helices arranged in layers and the alkyl side chains crystallized in a separate phase. PAALA-6 and PAALA-8 display an intermediate behavior according to the size of their side groups.[6]

In addition to the 13/4 helix, a second helical conformation also stabilized by intramolecular hydrogen bonds but containing four residues per turn has been described for both butyl and isobutyl poly(β-L-aspartate)s.[7,8] These helices are also right-handed and crystallize in a tetragonal structure in the $P4_1$ space group. The interconversion of the hexagonal form into the tetragonal form may be promoted either by heating or by immersion in light alcohols for a few minutes. The preference of each polymer for one form or the other seems to be largely determined by the chemical structure of the side group. In this regard it should be stressed that the tetragonal form has been observed neither for PAALA-*n* with *n* higher than 4 nor for poly(α-2-methoxyethyl β-L-aspartate), which has a side chain of size similar to that the butyl derivative but with one methylene replaced by oxygen.[9] Recent molecular mechanics calculations have shown that the relative importance of the two helical forms for a specific polymer may be accounted for by the side chain interactions taking place at both intra- and intermolecular levels.[10]

In spite of the fact that a good deal of knowledge has been accumulated on the structure of poly(β-L-aspartate)s, no structural information about members containing short alkyl side chains, *i.e.*, those with less than four carbon atoms, is available at present. The study of the lower members of the family is of interest because, according to what is known to happen in the related polypeptides poly(α-aspartate)s and poly(α-glutamate)s,[11,12] significant deviations from the standard structural behavior may be expected when the

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Table 1. Selected Data for the Poly(β-L-aspartarte)s −[HNCH(R)CH$_2$CO]− Studied in This Work

PAALA	R	M_0 (UCR)	[η] (dL g^{-1})[b]	$M_v \times 10^{-5}$ [c] I	II	D (g mL^{-1})[d]	T_d (°C)[e]	
PAALA-1[a]	CH$_3$	129.20	2.88	5.8	2.5	1.36	261	370
PAALA-2[a]	CH$_2$CH$_3$	143.22	2.66	5.3	2.3	1.26	261	368
PAALA-3	CH$_2$CH$_2$CH$_3$	157.26	6.19	14.0	7.1	1.11	261	367

[a] Data taken from ref 14. [b] Intrinsic viscosity measured in dichloroacetic acid at 23 °C. [c] Average viscosity molecular weights estimated by using the equations reported for poly(γ-benzyl α-L-glutamate)[15] (I) and for poly(γ-methyl α-L-glutamate)[16] (II). [d] Densities measured for samples coming directly from synthesis. [e] Melting−decomposition temperatures corresponding to imidation and main-chain scission determined by DSC−TGA.

length of the alkyl side group is reduced to minimum values. The precise conformational arrangement of the helix in the hexagonal form, the propensity to adopt the tetragonal structure, and the occurrence of extended conformations similar to the pleated β-sheet of polypeptides are some of the aspects strongly influenced by the constitution of the side group deserving particular attention. The present work is concerned with the analysis of the structure of poly(α-alkyl β-L-aspartate)s with the alkyl chain being methyl, ethyl, and propyl, which will henceforth be called PAALA-1, −2, and −3, respectively, according to the nomenclature established for the higher members of the series.[6] The characterization of the structure is made on the basis of X-ray diffraction and polarized infrared spectroscopy data. Crystal models are built and refined against experimental diffraction intensities by means of the LALS program supported by molecular mechanics energy calculations. The results are interpreted in terms of the constitution of the side chain and are critically discussed within the frame of the structural behavior outlined for the poly(β-L-aspartate) family.

Experimental Section

The three poly(β-L-aspartate)s used in this work were prepared by nonassisted anionic ring-opening polymerization of the corresponding (*S*)-4-(alkoxycarbonyl)-2-azetidinones. A detailed account of the synthesis of PAALA-1, PAALA-2, and their respective monomers has been reported elsewhere.[13,14] PAALA-3 was synthesized specifically for this work by applying the same methodology, and its chemical constitution was characterized by spectroscopic methods. A selection of data of the three polymers which are relevant to this study is given in Table 1. Although molecular weights estimated by viscosimetry are only approximate, it may be ascertained that we are dealing with high polymers having polymerization degrees of several thousands.

Densities were measured by the flotation method in either aqueous KBr solutions or carbon tetrachloride−toluene mixtures. The preparation of oriented samples in the helical form for polarized infrared spectroscopy was accomplished by applying the technique described by Ingwald[17] with some minor modifications. In brief, the polymer was incorporated in a film of poly(ethylene oxide) (PEO) and the composite film stretched at room temperature. Oriented films in the β form were prepared by casting from trifluoroethanol, cut into strips, and stretched several times their original length in boiling water. Infrared spectra were registered on a Perkin-Elmer 2000 FT-IR instrument equipped with a gold wire polarizer. To minimize errors due to the polarization monochromators, the orientation axis of the sample was placed at ±45° with respect to the entrance slit. Dichroic ratios (∥/⊥) of characteristic peaks were estimated from the absorbances measured for the parallel and perpendicular orientations of the film to the infrared polarization vector, and the corresponding minimum orientation factors were calculated according to Fraser.[18] A detailed description of the experimental details and calculations detailed in the polarized infrared analysis of poly(β-L-aspartate)s has recently been reported.[19]

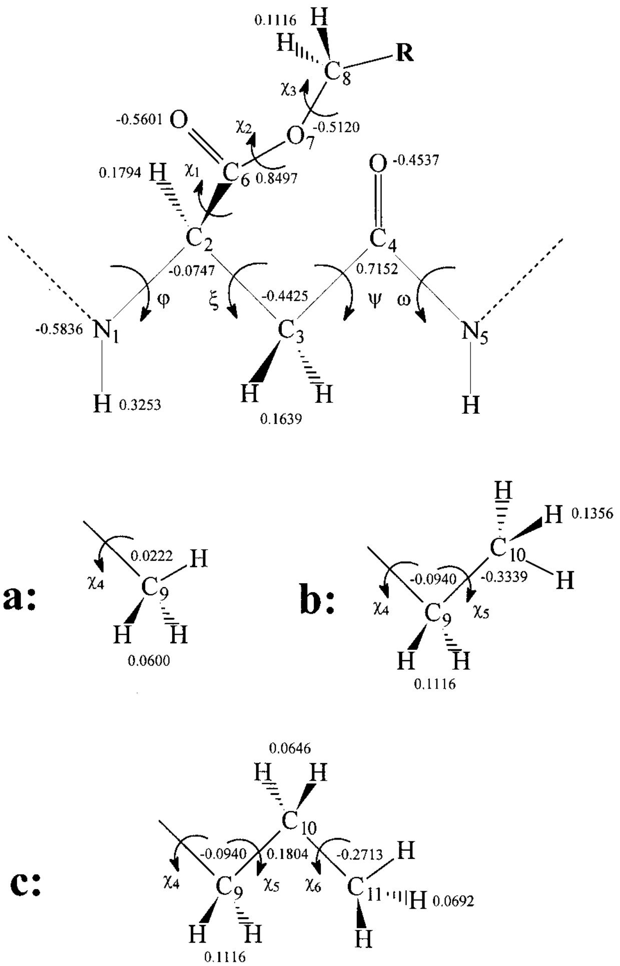

Figure 1. Dihedral angle notation and electrostatic charges computed for (a) poly(α-methyl β-L-aspartate) (PAALA-1), (b) poly(α-ethyl β-L-aspartate) (PAALA-2), and (c) poly(α-propyl β-L-aspartate) (PAALA-3).

A preliminary search for the helical conformations compatible with experimental X-ray diffraction data was performed using the GEMOX (GEneration of polymer molecular MOdels for X-rays) computer program.[20] This is a computational method based on a corrected grid search algorithm designed to generate all the molecular arrangements sterically allowed for a system with a given helical symmetry and an estimated axial repeating length. Molecular mechanics calculations were performed with explicit consideration of all atoms using the AMBER 3.0 Rev.A program[21] with all-atom parametrization.[22] Parameters previously developed by us for other poly(β-L-aspartate)s were used.[10,23] Dihedral angle notation and atomic charges for the monomeric unit of the three poly(β-L-aspartate)s included in this study are given in Figure 1.

X-ray diffraction diagrams were obtained on flat films in a Statton-type camera (W. H. Warhus, Wilmington, DE) using nickel-filtered copper radiation of wavelength 1.542 Å and calibrated with molybdenum sulfide (d_{002} = 6.147 Å). Samples were prepared from cast films which were stretched under a variety of conditions including heating and immersion in appropriate solvents. X-ray diffraction data were used for the analysis of the crystal structure by the LALS (linked-atom least squares) program.[24] The reflection intensities were estimated with a unidimensional Joyce Loebl MKIII microdensitometer. Layer lines were unidirectionally scanned, and the integrated areas under each spot were measured by weighing and corrected to take into account the orientation of the diffracting planes. Intensities were also corrected for polarization and Lorentz factors. No absorption correction was needed since the films used for diffraction were thin. Multiplicity corrections were also made when required. Standard bond

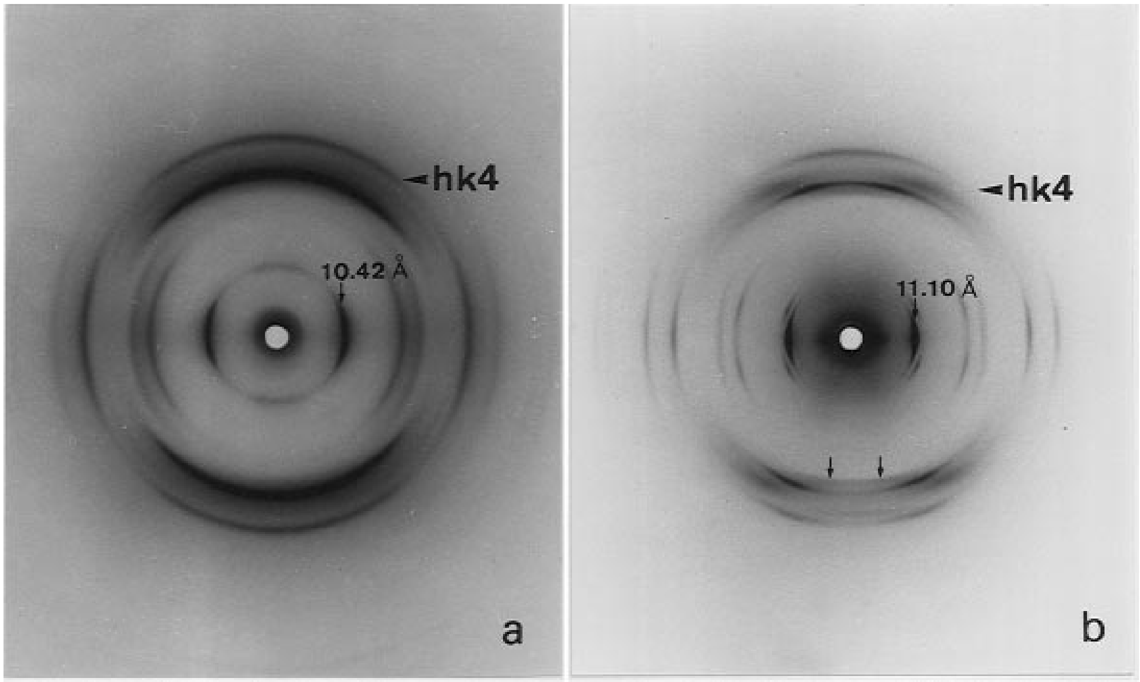

Figure 2. X-ray diffraction patterns of oriented films of PAALAs in the hexagonal form: (a) PAALA-1 cast from hexafluoro-2-propanol and stretched 200% at room temperature; (b) PAALA-3 cast from a $CHCl_3$−trifluoroacetic acid mixture and stretched about 300% at room temperature. The stretching direction is vertical in both cases.

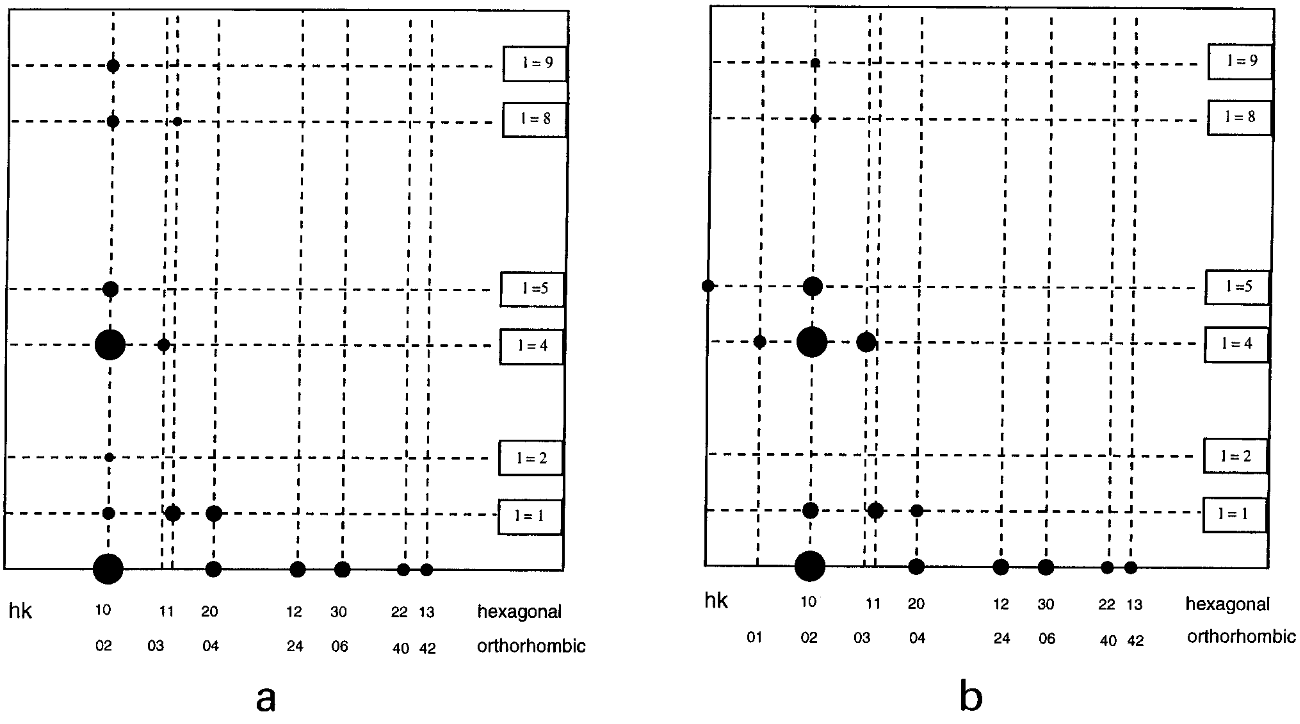

Figure 3. Reciprocal lattice rotation diagrams for the hexagonal form of PAALA- 1 (a) and PAALA-3 (b). Reflection intensity is approximately represented by the size of the circle.

lengths and angles were kept fixed, and the torsion angle ω for the amide group was held at 180°, corresponding to the *trans* conformation. Hydrogen bonds were assumed to be almost linear with an approximate length of 2.90 Å. Chain conformation and crystal packing were then refined against X-ray intensities so that close contacts were minimized at both intra- and intermolecular levels.

Results

A. The Hexagonal Form. (1) X-ray Diffraction and Infrared Spectroscopy. Films of PAALA-1 and PAALA-2 prepared by casting from hexafluoro-2-propanol and subsequently stretched about 200% at room temperature produced the same type of diffraction pattern, which is illustrated in Figure 2a for the case of the methyl derivative. The scattering along the equator of these diagrams is consistent with a hexagonal packing of helices of diameter 12.03 and 12.67 Å for PAALA-1 and PAALA-2, respectively. On the other hand, the off-equatorial reflections were found to be arranged in a series of layer lines which enables one to fix the chain axis repeat of the structure at a value of 20.5 Å for both polymers. A schematic indexing of the diffraction signals observed in the experimental diagram is depicted in Figure 3a, and the interplanar spacings

Table 2. Observed and Calculated Spacings (Å) for the Hexagonal Form of PAALAs

	PAALA-1		PAALA-2		PAALA-3		hkl^e	
layer line	d_{hkl}(obsd)[a]	d_{hkl}(calcd)[b]	d_{hkl}(obsd)[a]	d_{hkl}(calcd)[c]	d_{hkl}(obsd)[a]	d_{hkl}(calcd)[d]	HEX	ORTH
ll = 0	10.40 vs	10.42	10.87 vs	10.97	11.10 vs	11.10	100	110, 020
	5.20 m	5.21	5.40 m	5.48	5.54 m	5.55	200	220, 040
	3.91 m	3.94	4.10 m	4.15	4.17 m	4.20	120	310, 240, 150
	3.46 m	3.47	3.61 m	3.66	3.69 m	3.70	300	330, 060
	3.00 m	3.01	3.12 w	3.17	3.19 w	3.20	220	260, 400
	2.87 w	2.89	3.00 w	3.04	3.05 w	3.08	130	420, 350, 170
ll = 1	9.35 w	9.29	9.44 vw	9.68	9.96 m	9.85	101	111, 021
	5.84 m	5.77	6.08 m	6.05	6.24 m	6.14	111	201, 131
	5.09 m	5.05	5.27 m	5.30	5.41 w	5.37	201	221, 041
ll = 2	7.47 vw	7.31					102	112, 022
ll = 4	4.58 vs				5.18 w	5.20	104	014
		4.60	4.63 vs	4.65	4.78 vs	4.81		114, 024
	4.10 w	4.13	4.23 w	4.20	4.36 s	4.33		034
ll = 5	3.82 m				4.24 w	4.28	005	005
		3.82	3.87 m	3.84	4.00 s	3.99	105	115, 025
ll = 8	2.48 w	2.49	2.50 w	2.50	2.55 vw	2.60	108	118, 028
	2.35 vw	2.36				2.62		108
ll = 9	2.22 w	2.23	2.23 w	2.23	2.33 vw	2.32	109	119, 029

[a] Visually estimated intensities: vs, very strong; s, strong; m, medium; w, weak; vw, very weak. [b] Calculated from a hexagonal unit cell of parameters: a = 12.03 Å, c = 20.51 Å (monoclinic, a = 12.03 Å, b = 20.84 Å, c = 20.51 Å). [c] Calculated from a hexagonal unit cell of parameters: a =12.67 Å, c = 20.51 Å (monoclinic, a = 12.67 Å, b = 21.94 Å, c = 20.51 Å). [d] Calculated from a hexagonal unit cell of parameters: a = 12.82 Å, c = 21.38 Å (monoclinic, a = 12.82 Å, b = 22.20 Å, c = 21.38 Å). [e] Indexing for the pseudohexagonal unit cell containing one chain and for the monoclinic cell containing two chains pointing out to opposite directions.

observed and calculated for each polymer are listed in Table 2. Although no clear evidence requiring a larger unit cell was found, indexing for a monoclinic structure containing two chains arranged in opposite directions, similar to the structure described for the hexagonal form of other poly(β-L-aspartate)s, [5−7] is given as well.

Further evidence on the α-helix-like conformation present in these compounds was furnished by polarized infrared spectroscopy. The infrared spectra of stretched films of PAALA-1 and PAALA-2 registered with the polarization vector parallel and perpendicular to the stretching direction are shown in Figure 4. Similar results are obtained for the two polymers. Estimates of dichroic ratios, D, as well as the calculated minimum orientation factors, $f_{\min}$, for the amide characteristic bands are given in Table 3. As expected from the hydrogen bond orientation taking place in a structure of α-helix type, a parallel dichroism is exhibited by both the amide A (3280−3285 cm^{-1}) and the amide I (1654 cm^{-1}) bands, which is the dichroic character typically observed for the α-helix of poly(α-amino acids).[11,12] The parallel dichroism shown by the amide II band (1541−1542 cm^{-1}) is however contrary to what should be expected for such conformation. This discrepancy, which has been shown to be due to the different inclination adopted by the amido group with respect to the chain axis,[19] appears to be shared by all poly(β-L-aspartate)s in the 13/4 helical conformation.[6]

A fiber of PAALA-3 obtained by pulling out a jelly of polymer in chloroform provided the X-ray diffraction pattern shown in Figure 2b. A schematic representation of the experimental diagram is given in Figure 3b, where a close comparison with the scheme corresponding to PAALA-1 (which is practically indistinguishable from that obtained for PAALA-2) is straightforward. The pattern is substantially identical to that reported for the hexagonal form of poly(α-isobutyl β-L-aspartate),[5] and it has therefore been interpreted in terms of a similar structure composed of right-handed 13/4 helices with a diameter of 12.82 Å and an axial repeat of 21.4 Å. A close inspection of the pattern reveals that the meridional reflection on the fourth layer line is split, disclosing that the true unit cell contains at least two

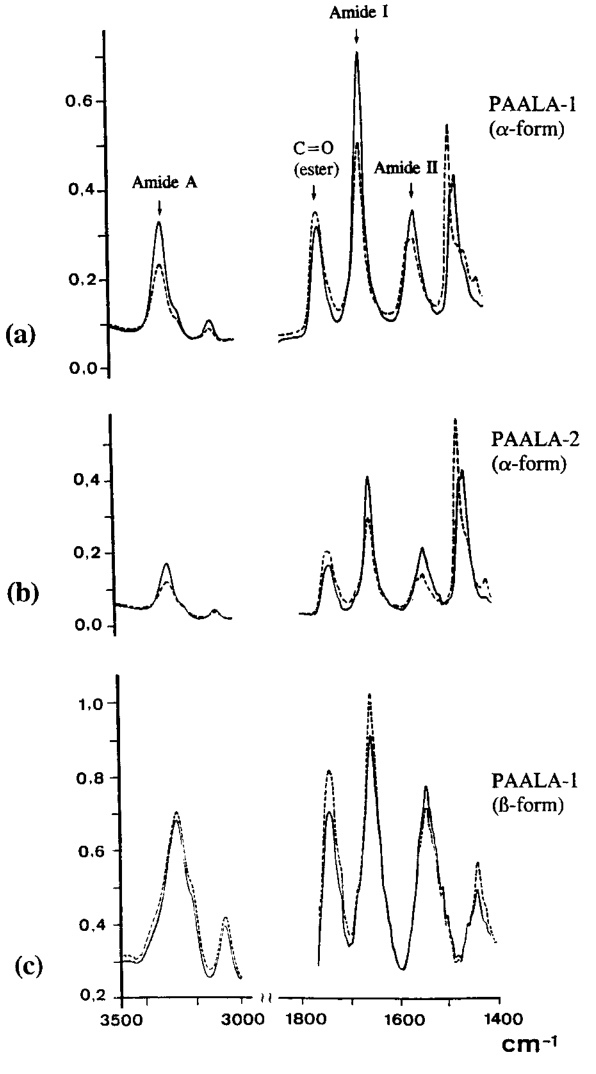

Figure 4. Dichroic infrared spectra of stretched films of PAALAs in the 3500−3000 and 1800−1400 cm^{-1} regions: (a) PAALA-1 in the hexagonal form; (b) PAALA-2 in the hexagonal form; (c) PAALA-1 in the β form. Spectra were measured with the electric vector vibrating perpendicular (broken line) and parallel (full line) to the stretching direction.

Table 3. Polarizing Infrared Parameters for the Hexagonal Form and the β Form of PAALAs

		band position (cm^{-1}) and dichroic ratio (D)								
		amide A		amide I		amide II		CdO ester		
polymer	crystal form	cm^{-1}	$D(\parallel/\perp)$	cm^{-1}	$D(\parallel/\perp)$	cm^{-1}	$D(\parallel/\perp)$	cm^{-1}	$D(\parallel/\perp)$	$f_{\min}$[a]
PAALA-1	hexagonal	3280	1.56	1654	1.64	1541	1.56	1745	0.95	0.18
	β-form	3277	0.99	1656	0.85	1543	1.05	1743	0.87	0.11
PAALA-2	hexagonal	3284	1.65	1654	1.57	1542	2.06	1742	0.85	0.34

[a] Minimum orientation factor calculated according to Fraser.[18]

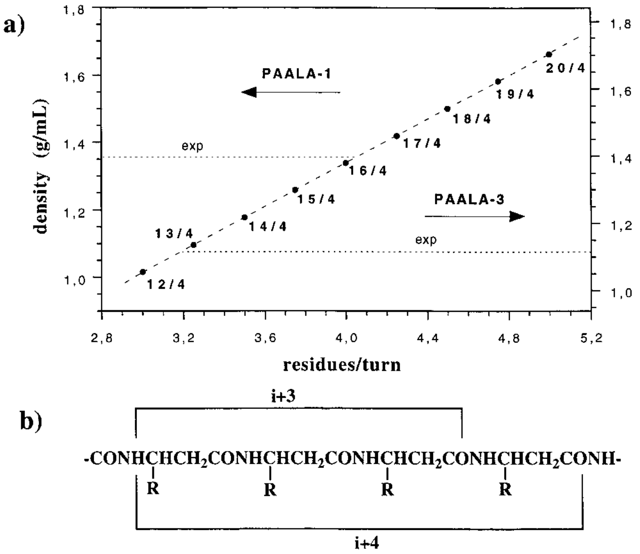

Figure 5. (a) Calculated density plotted as a function of the number of residues per turn of the helix of PAALA-1 and PAALA-3 in the hexagonal form. (b) Hydrogen-bonding schemes compatible with the helical conformation of PAALAs.

molecules. Accordingly, the pattern should be indexed on the basis of a monoclinic unit cell of parameters a = 12.82 Å, b = 22.20 Å, and c = 21.38 Å. The observed and calculated spacings for this form along with the indexing considering both the pseudohexagonal and the monoclinic cells are given in Table 2 together with the corresponding data of PAALA-1 and PAALA-2. The density calculated for this structure is 1.12 g mL^{-1}, in excellent agreement with the value obtained experimentally, which is 1.11 g mL^{-1}.

(2) Energy Calculations. The strong intensity displayed by the fourth layer line in X-ray diagrams of this form leads one to interpret the structure in terms of a helical repeating unit consisting of four turns. On the other hand, density measurements indicate that more than 3.5 residues per turn are required to attain an acceptable agreement between experimental and calculated values. In Figure 5a, the density calculated for the hexagonal structure of PAALA-1 and PAALA-3 is plotted as a function of the number of residues per turn of the helix. It can be seen that the experimental density of PAALA-1 falls between the values estimated for the 15/4 and 17/4 helices, having 3.75 and 4.25 residues per turn, respectively, which appear to be the only reasonable models for this polymer. Similar conclusions may be drawn for PAALA-2 when density considerations are taken into account, although in this case the experimental density is much closer to that calculated for the 15/4 helix. A complete survey of molecular models compatible with such helices was carried out with the GEMOX program.[20] Twelve and nine models were feasible for the 15/4 and the 17/4 helices, respectively. All these models were right-handed and correspond to hydrogen-bonding schemes entailing the i residue linked either to the $i + 3$ or $i + 4$ residue as shown in Figure 5b.

Molecular mechanics calculations were then performed on both PAALA-1 and PAALA-2 using as starting models all those accepted by GEMOX. In both cases, conformations with 17/4 symmetry were found to be energetically favored with respect to those with 15/4 symmetry by at least 3.5 kcal mol^{-1} residue^{-1}. Moreover, hydrogen bond geometry for 15/4 helices appeared to be significantly distorted in both angles and distances. The effects of crystal environment on conformation were investigated for 17/4 models having relative energies between 0 and 2.5 kcal mol^{-1}. For this, hexagonal arrays of helices in each selected conformation were built in both parallel and antiparallel arrangement following the same methodology previously used by us for PAALA-4.[10] The minimum energy for the two polymers was found to correspond to an antiparallel structure with chains rotated 180° around each other. Such arrangement results in a monoclinic lattice in the space group $P2_1$ similar to that described for other poly(β-L-aspartate)s.[5,10] The dihedral angles resulting from these calculations for the most favored model for each polymer are given in Table 4.

(3) Modeling and Refinement by LALS. The minimum energy conformations obtained in the preceding section were used for building the models of PAALA-1 and PAALA-2 to be analyzed by LALS. Backbone and side chain dihedral angles were first refined under the constraints required to preserve helix continuity and hydrogen-bonding scheme. The helical geometry was then refined by introducing packing constraints relative to experimentally determined crystal parameters and lattice symmetry. Changes in the backbone dihedral angles resulting from this step were small, the largest variation observed being 13°. Finally, chain packing was refined against X-ray reflection intensities. Observed and calculated structure factors are compared in Table 5, which shows that a good concordance has been achieved as reflected in the R factors of 14.8 and 15.7% obtained for PAALA-1 and PAALA-2, respectively. The dihedral angles and hydrogen-bonding geometry calculated for the refined hexagonal form of each polymer are given in Table 4. A representation of the resulting 17/4 helix is depicted in Figure 6, and the projection down the chain axis of the $P2_1$ unit cell is shown in Figure 7. The resulting structure consists of a crystal lattice similar to that known for the hexagonal form of both poly(α-n-butyl β-L-aspartate)[7] and poly(α-isobutyl β-L-aspartate)[5] but with chains in a helical conformation closer to the 4/1 helix characteristic of the tetragonal form of these polymers.

The model for the hexagonal form of PAALA-3 was initially built using the conformational parameters reported for the right-handed 13/4 helix of poly(β-L-aspartate)s by means of molecular mechanics calculations. Such data are given in Table 4 for comparison. The model was then refined by LALS, first on a stereochemical basis and then against experimental

Table 4. Conformational Parameters and Hydrogen Bond Geometries for the Helical Forms of PAALAs

	PAALA-1 (HEX)		PAALA-2 (HEX)		PAALA-2 (TET)		PAALA-3 (HEX)	
	MM[a]	LALS[b]	MM	LALS	MM	LALS	MM	LALS
torsional angles (deg)								
ϕ	135.1	141.7	147.0	141.6	135.4	144.2	145.4	144.0
ξ	−87.1	−92.3	−95.8	−92.3	−63.9	87.0	−59.7	−59.7
ψ	157.0	155.4	141.8	155.4	144.7	148.2	125.9	132.4
ω	169.1	180.0	174.3	180.0	162.4	180.0	−178.0	180.0
χ_1	179.5	145.8	165.2	147.9	−177.2	147.6	−175.9	145.0
χ_2	173.2	180.0	176.2	180.0	−152.4	180.0	174.1	180.0
χ_3			168.3	180.0	178.8	180.0	180.0	180.0
χ_4							−159.4	180.0
H-bond geometry								
d(H···O) (Å)	2.14	2.20	2.17	2.20	1.75	1.81	1.89	2.03
d(N···O) (Å)	3.00	3.10	3.06	3.10	2.75	2.82	2.81	3.02
∠NHO (deg)	141.9	160.6	149.6	160.5	171.2	178.2	149.9	174.7
R-factor (%)		14.8		15.7				13.3

[a] Molecular mechanics calculations. [b] Linked-atom least-squares calculations.

Table 5. Observed and Calculated Structure Factors (*F*) for the Hexagonal Crystal Form of PAALAs

		PAALA-1				PAALA-2				PAALA-3			
spot	*hkl*[a]	d_{hkl}(obs)	F_o	F_c	$F_o - F_c$	d_{hkl}(obs)	F_o	F_c	$F_o - F_c$	d_{hkl}(obs)	F_o	F_c	$F_o - F_c$
1	020 110	10.40	237.4	213.7	23.66	10.87	229.2	206.3	22.90	11.1	285.8	315.6	−29.9
2	040 220									5.54	80.2	88.1	−79
3	150 240 310	3.91	253.0	222.0	31.03	4.10	251.3	220.9	30.42	4.17	112.9	148.5	−35.6
4	060 330	3.46	186.5	171.7	14.78	3.61	177.8	161.1	16.71	3.69	94.2	99.8	−5.6
5	260 400	3.00	136.1	177.3	−41.24	3.12	133.5	178.4	−44.87	3.19	99.0	86.3	12.7
6	170 350 420									3.05	102.8	98.0	4.8
7	021 111									9.96	68.4	97.5	−29.1
8	131 201	5.84	119.4	110.2	9.18	6.08	126.9	149.3	22.43	6.24	130.0	123.6	6.7
9	041 221	5.09	109.1	116.7	−7.57	5.27	114.4	118.5	−4.05	5.41	103.8	94.1	9.7
10	024 114	4.58	286.9	223.5	63.45	4.63	274.1	201.5	72.57	4.81	161.0	142.4	18.6
11	025 115	3.82	89.9	70.8	19.10	3.87	92.3	61.6	30.71	4.00	139.7	122.5	17.2
12	028 118									2.55	95.6	114.6	19.0

[a] Indexed on the basis of the monoclinic unit cell.

X-ray diffraction data. As expected, the resulting 13/4 helix is substantially identical to that described for poly(α-isobutyl β-L-aspartate). A schematic representation of this helix is shown in Figure 6. Note that in this case hydrogen bonding takes place between residues *i* and *i* + 3.

B. The Tetragonal Form. (1) X-ray Diffraction. The X-ray diagrams of the tetragonal form previously described for poly(β-L-aspartate)s with *n*-butyl[7] and isobutyl[8] side chains are characterized by the presence of a strong reflection spacing of 14.15 and 14.0. Å, respectively, which correspond to the diameter of the 4/1 helix adopted in each case. This structure is usually observed in powdered samples precipitated by light alcohols as well as in unoriented films prepared by casting from 2-chloroethanol. A film of PAALA-2 obtained by the latter method produced a diffuse Debye–Scherrer diagram containing a ring with a Bragg spacing around 12.5 Å, close to what should be expected for this polymer crystallized in the tetragonal form. When such film was stretched at 120 °C and left under the applied stress for a few days, the oriented pattern shown in Figure 8 could be obtained. As shown in Table 6, the whole pattern may be indexed on the basis of a tetragonal unit cell of parameters $a = 12.30$ Å and $c = 4.90$ Å containing one chain. The density calculated for this structure is 1.29 g mL^{-1}, in agreement with the value of 1.28 g mL^{-1} found experimentally.

The behavior displayed by PAALA-3 with regards to formation of the tetragonal form was similar to that observed for PAALA-2. The film prepared from 2-chloroethanol gave poorly defined diagrams with a ring spacing around 13 Å, close to the value that should be expected for the 4/1 helix of a poly(β-L-aspartate) bearing an *n*-propyl side group. When this film was subjected to treatments known to favor the crystallization in the tetragonal form, mixtures of the tetragonal and hexagonal phases in different proportions and with varying degrees of ordering were obtained. With respect to PAALA-1, all attempts made to obtain this polymer in the tetragonal form were unsuccessful. Samples were subjected to a combination of treatments including stretching, heating, and dipping in solvents known to promote the formation of the tetragonal structure. It was invariably found however that either the initial hexagonal form was retained or a new crystalline form

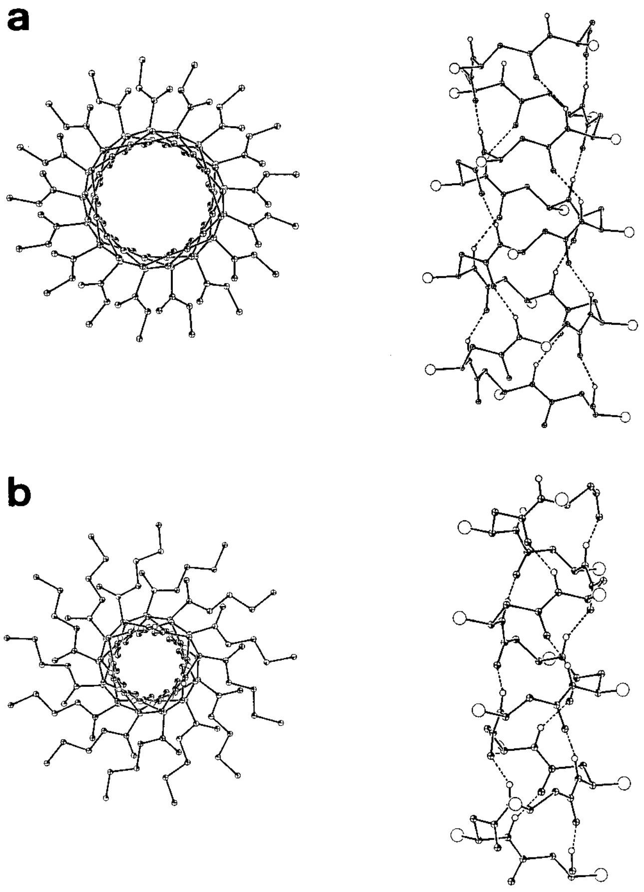

Figure 6. Axial and equatorial projections of the 17/4 helix of PAALA-1 (a) and the 13/4 helix of PAALA-3 (b). In the axial views, the side groups are indicated by spheres in order to make clearer the representation.

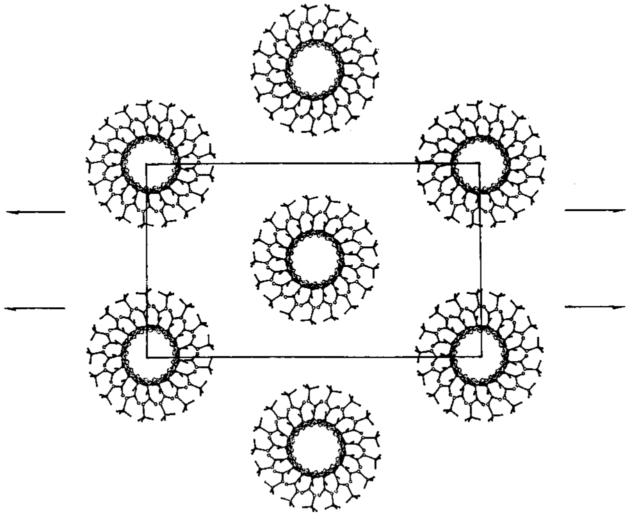

Figure 7. Projection along the *c* axis of the unit cell of the hexagonal form of PAALA-1 obtained by combining energy calculations and LALS refinement.

appeared, which was later identified as a structure made of extended chains. Thus we may reasonably conclude that PAALA-1 is distinguished from PAALA-2, -3, and -4 in being unable to adopt the 4/1 helical conformation.

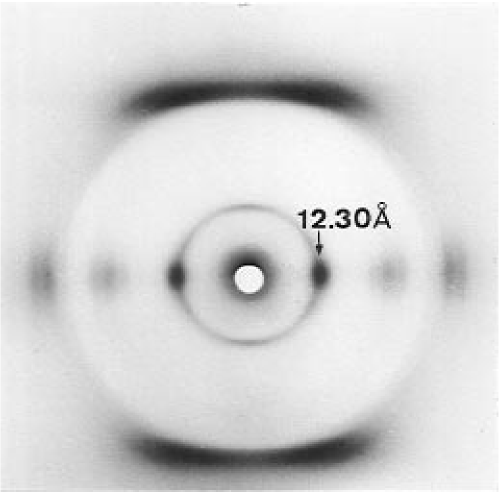

Figure 8. X-ray diffraction pattern of PAALA-2 in the tetragonal form prepared by stretching at 120 °C a film cast in 2-chloroethanol. The stretching direction is vertical.

Table 6. Observed and Calculated Spacings (Å) for the Tetragonal Form of PAALA-2

layer line	d_{hkl}(obsd)[a]	d_{hkl}(calcd)[b]	*hkl*
ll = 0	12.29 vs	12.30	100
	6.09 m	6.15	200
	5.56 m	5.50	210
	4.38 s	4.35	220
	4.10 m	4.10	300
	3.91 vw	3.89	310
	3.44 w	3.41	320
	3.07 w	3.08	400
ll = 1	4.60 vs	4.55	101
	4.27 s	4.27	111
ll = 2	2.46 w	2.45	002
		2.40	102

[a] Visually estimated intensities: vs, very strong; s, strong; m, medium; w, weak; vw very weak. [b] Calculated on the basis of a tetragonal unit cell of parameters: $a = 12.30$ Å, $c = 4.90$ Å.

(2) Energy and LALS Calculations. Molecular mechanics calculations for the tetragonal form of both PAALA-2 were performed using the parameters reported for the right-handed 4/1 helix of PAALA-4. The crystal environment was mimicked in order to take into account the influence of nonbonding interactions between neighboring chains. A parallel arrangement of helices with the same orientation around the chain axis corresponding to a space group $P4_1$ was found to be the most stable mode of packing. For consistency with previous work, the model resulting from these calculations was subjected to analysis by LALS. This was carried out on the exclusive basis of stereochemical grounds since similarities with the model described for PAALA-4 made it unnecessary to refine against experimental data. The dihedral angles of both the energetically minimized model and the crystal model resulting from LALS are given in Table 4. The axial representation of this helix and the projection of the unit cell along the *c* axis are shown in Figure 9.

C. The β Form. A film of PAALA-1 in the hexagonal form stretched in boiling water produced the diagram shown in Figure 10a, which is clearly dissimilar to any type of diagram recorded up to date for poly(β-L-aspartate)s. Distinct features of the diagram are the absence of layer lines corresponding to spacings larger than 4.6 Å and a distribution of the scattering along the equator incompatible with either a tetragonal or a hexagonal packing of helices. On the contrary, the overall pattern appears to be in satisfactory agreement with that expected for an orthogonal structure composed of up and down chains in a nearly extended conformation. The axial repeat of this structure measured on the first and second layer lines is about 4.6 Å, indicating

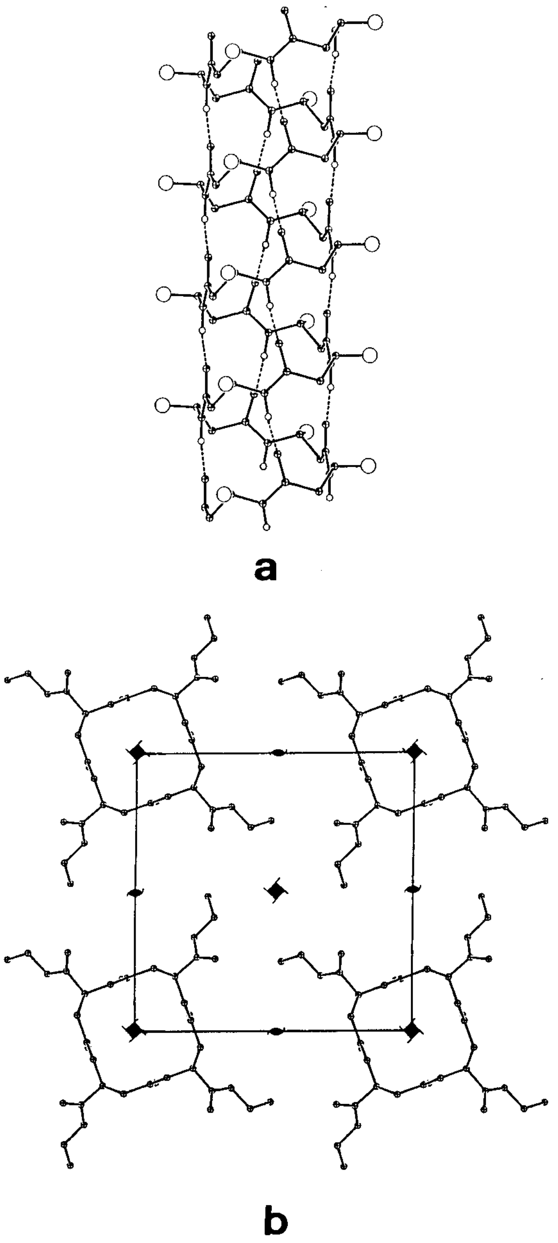

Figure 9. (a) Axial projection of the 4/1 helix of PAALA-2. (b) Projection along the *c* axis of the unit cell of the tetragonal form of PAALA-2 obtained by combining energy calculations and LALS refinement.

a contraction of the chain to an extent similar to that occurring in the β form of polypeptides.[11,12] The equatorial reflections with spacings 4.6 and 7.5 Å are interpreted as corresponding to the interchain and interlayer distances respectively of a structure composed of hydrogen-bonded sheets. As shown in Table 7, the pattern may be satisfactorily indexed on the basis of a metrically orthorhombic unit cell of parameters $a = 9.16$ Å, $b = 14.96$ Å, and $c = 4.61$ Å containing four chains. The density calculated for this structure is 1.36 g mL^{-1}, which is practically identical to the density determined experimentally.

Polarized infrared spectroscopy furnished additional evidence to distinguish between the helical and extended forms of PAALA-1. Whereas no significant differences in the position of the characteristic bands were detected, the dichroic sign of some of such bands was found to be reversed. The infrared dichroic spectra arising from the hexagonal and the β forms for the parallel and perpendicular orientations of the radiation vector are compared in Figure 4, and the estimated spectral parameters are listed in Table 3. The opposite dichroic character observed for both the amide A and the amide I bands leaves no doubt about the orientation of the amide group with respect to the molecular axis in the two forms. According to basic principles of polarized infrared spectroscopy and antecedents on polypeptides,[11,12] the orientation of the amide group in the β form must be such that the NH and CO stretching deformations take place in a direction not far from the normal to the fiber axis. This is in full concordance with the molecular arrangement occurring in the structural model given for this form on the basis of the X-ray analysis.

We also searched for the β form in PAALA-2 and PAALA-3 by stretching these polymers under the action of water. Whereas no traces of the β form were detected for PAALA-3, the results attained in the case of PAALA-2 were much like those obtained for PAALA-1. The X-ray diffraction pattern of a film of PAALA-2 oriented in boiling water is reproduced in Figure 10b. The pattern corresponds to the polymer in the β form but contaminated with a considerable amount of tetragonal form. As shown in Table 7, the nine reflections arising from the β form were consistent with a metrically orthorhombic unit cell of dimensions $a = 9.16$ Å, $b = 16.82$ Å, and $c = 4.61$ Å (fiber axis) containing four chains. The structure is similar to that described above for the β form of PAALA-1, the only difference being concerned with the interlayer distance as logically expected from the increasing in size of the alkyl side group.

Discussion

The results obtained in this study reveal that PAALA-1 and PAALA-2 present significant structural novelties with respect to what has been recognized by now to be the standard pattern of poly(β-L-aspartate)s. Conversely, PAALA-3 displays a structural behavior substantially similar to that of PAALA-4 with no conformational differences worthy of mention. The most relevant parameters of the crystal forms observed for the three PAALAs studied in this work are collected in Table 8, where data reported for PAALA-4 have also been included for comparison.

The occurrence of crystal polymorphism involving helical and extended conformations is a well-known fact among polypeptides, in particular among esters of the poly(α-L-glutamic acid) and poly(α-L-aspartic acid). For instance, poly(β-benzyl α-L-aspartate) may adopt two helical forms, known as the α and ω forms, respectively, in addition to the pleated sheet β form.[25,26] It is also known that the preference for the extended conformation is enhanced as the size of the side group decreases. The results presented in this work together with those obtained in previous studies reported on poly(β-L-aspartate)s bearing larger alkyl side chains definitely evidence the close similarity existing in the structural behavior of poly(β-aspartate)s and poly(α-amino acid)s.

The helical arrangement found for the hexagonal form of PAALA-1 and PAALA-2 has precedents neither in poly(β-L-aspartate)s nor in polypeptides. A close inspection of the dihedrals resulting for the 17/4 helix indicates that this conformation is topologically close to the 4/1 helix. This makes much sense since the same hydrogen-bonding scheme made of hydrogen bonds set between the *i* and $i + 4$ residues has been used to construct the two helices. Although the 17/4 helix displays the same crystal packing preferences as the 13/4 helix, the twist

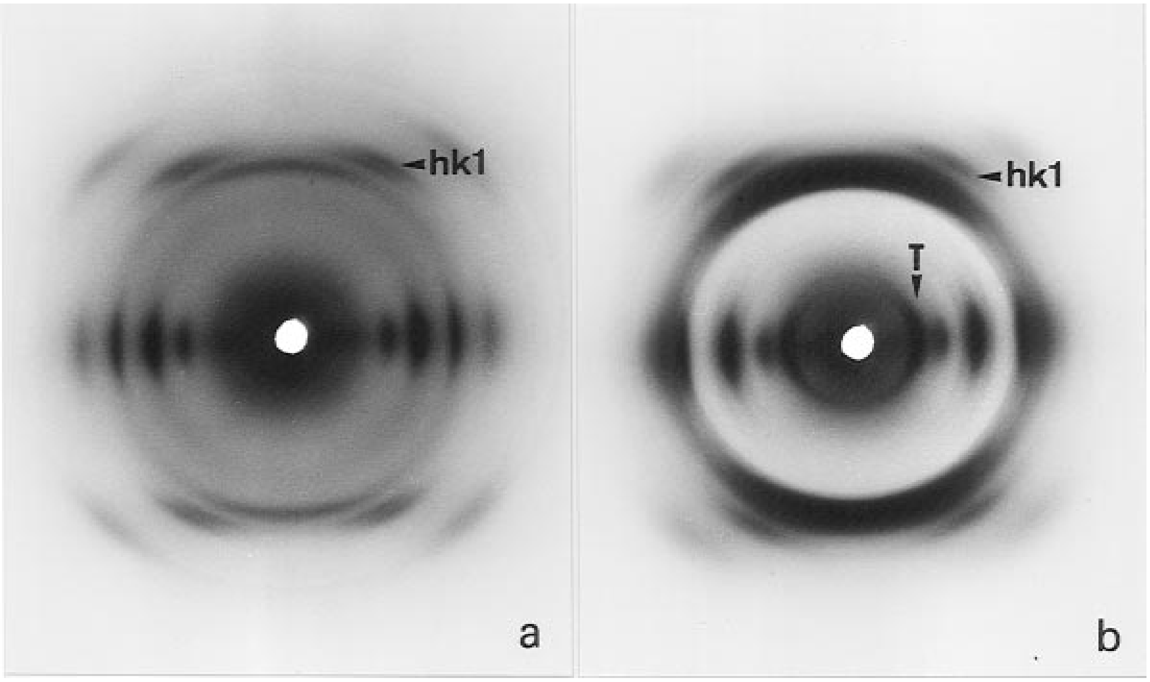

Figure 10. X-ray diffraction patterns of (a) PAALA-1 and (b) PAALA-2 in the β form. In the second case, a certain amount of tetragonal phase is observed to be still present. The stretching direction is vertical in both diagrams.

Table 7. Observed and Calculated Spacings (Å) for the β-Form of PAALA-1 and PAALA-2

	PAALA-1		PAAKA-2		
layer line	d_{hkl}(obsd)[a]	d_{hkl}(calcd)[b]	d_{hkl}(obsd)[a]	d_{hkl}(calcd)[c]	*hkl*
ll = 0	7.48 s	7.48	8.41 m	8.41	020
	5.74 vs	5.79	6.15 vs	6.19	120
	4.58 s	4.58	4.58 s	4.58	200
	3.87 m	3.90	4.08 s	4.02	220
ll = 1	4.50 m	4.61	4.50 s	4.61	001
		4.41		4.45	011
	4.12 m	4.12			101
	3.91 m	3.92	3.84 s	4.04	021
	3.23 m	3.25	3.23 m	3.25	201
ll = 2	2.29 w	2.30	2.33 w	2.30	002
		2.28		2.28	012
			2.24 w	2.23	102

[a] Visually estimated intensities: vs, very strong; s, strong; m, medium; w, weak. [b] Calculated for a metrically orthorhombic lattice of parameters: a = 9.16 Å, b = 14.96 Å, c = 4.61 Å. [c] Calculated for a metrically orthorhombic lattice of parameters: a = 9.16 Å, b = 16.82 Å, c = 4.61 Å.

Table 8. Crystal Forms of PAALA-*n*, $n \leq 4$

					density (g mL^{-1})
	helix	lattice	unit cell parameters (Å)	obsd	calcd
PAALA-1	17/4	HEX	$a = 12.03$, $c = 20.51$	1.36	1.42
	β	ORTH	$a = 9.16$, $b = 14.96$, $c = 4.61$	1.36	1.36
PAALA-2	17/4	HEX	$a = 12.67$, $c = 20.51$	1.26	1.41
	4/1	TET	$a = 12.30$, $c = 4.90$	1.28	1.28
	β	ORTH	$a = 9.16$, $b = 16.82$, $c = 4.61$	1.27	1.31
PAALA-3	13/4	HEX	$a = 12.82$, $c = 21.38$	1.11	1.12
	4/1	TET	$a = 13.29$, $c = 4.90$		
PAALA-4[a]	13/4	HEX	$a = 13.40$, $c = 20.60$	1.17	1.18
	4/1	TET	$a = 14.10$, $c = 4.90$	1.18	1.17

[a] Data taken from ref 9.

angle changes from 110.8 to 84.7°, approaching the 90° characteristic of the tetragonal form. It should be noted that the helical structures observed for polypeptides move within a much narrower range of twist angle values. A survey of the conformational data experimentally obtained for more than 20 different polypeptides[12] in the α form reveals oscillations of the unit twist of less than 3%, around 100°, which is the value for the standard 18/5 helix. On the other side, the ω form is described for various polypeptides as an almost perfect 4/1 helix with a twist angle of 901. Obviously, it is the presence of the additional methylene in the main chain which determines the higher conformational flexibility displayed by poly(β-aspartate)s when compared to polypeptides.

The tetragonal form characterized for PAALA-2 seems to be identical to that described earlier for both the *n*-butyl and isobutyl esters. The arrangement of the ethyl side chains turns out to be similar to that found for the *n*-butyl chains in the case of PAALA-4.[10] As a consequence of the decreasing in size of the alkyl group, crystallization of the PAALA-2 helices results in a more efficiently packed lattice, with the central hole characteristic of this structure being less prominent. According to this reasoning, the fact that the 4/1 helix was not observed for PAALA-1 may seem rather striking. However, molecular mechanics studies[10] have demonstrated that the interaction among side chains plays a decisive role in the unstabilization of the hexagonal form in favor of the tetragonal form. On the other hand, the behavior of PAALA-3 with regard to formation of the tetragonal form may be considered to be halfway between PAALA-2 and PAALA-4 as expected.

The handedness of the helical forms of poly(α-L-aspartate)s has been an issue of controversy which is not yet resolved.[27,28] Right- and left-handed helices have been proposed for poly(α-L-aspartate)s differing only in the constitution of the side chain and even for samples of the same polymer prepared under different conditions. The 4/1 and 13/4 helices described for all the poly(β-L-aspartate)s studied so far are reported to be right-handed, which is actually opposite to the sense that one should expect from the stereochemistry of the parent β-amino acid. The modeling analysis carried out with PAALA-1 and PAALA-2 has led to assignment of a right sense to the 17/4 helix too, and energy calcula-

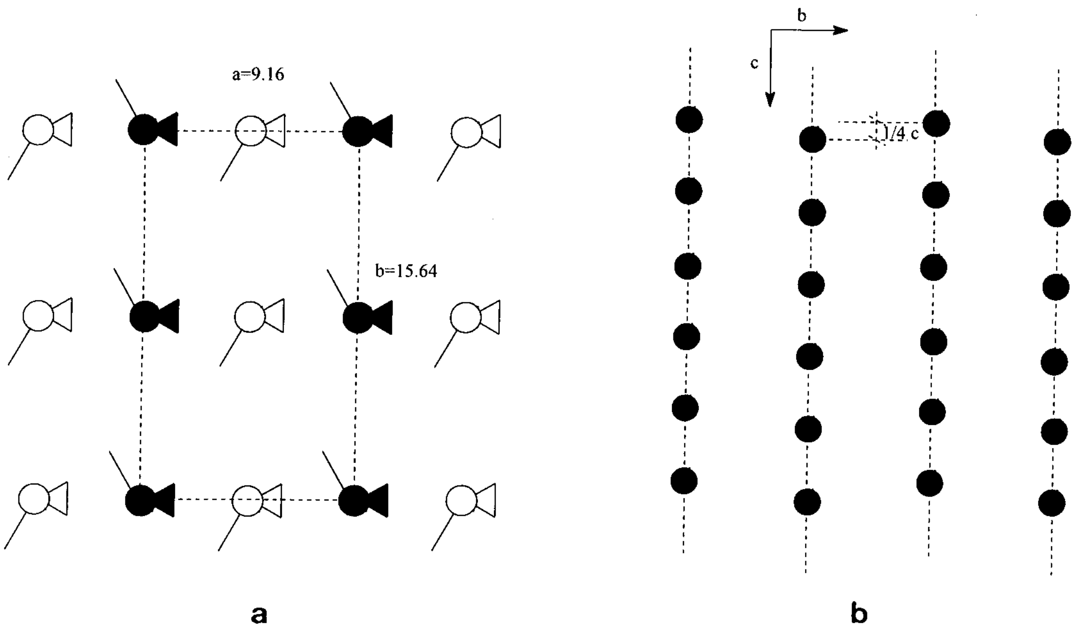

Figure 11. Schematic representation of the mode of packing in the crystal *β* form proposed for both PAALA-1 and PAALA-2: (a) view down the chain axis; (b) view along the *a* axis showing the alternating up-and-down staggering of the hydrogen-bonded sheets.

tions indicated that intramolecular amide–ester interactions should be taken into account to better explain such preference. Recent theoretical energy calculations carried out on a series of model compounds of poly(α-isobutyl *β*-L-aspartate)[29] concluded that a similar effect must operate in the stabilization of the 13/4 helix with a right sense.

This is the first time that a crystal form composed of chains in an extended conformation is described for a poly(*β*-L-aspartate). It has been necessary to reduce the size of the side chain up to two carbon atoms to render the extended arrangement observable. On the contrary, the propyl group seems to be still too large to allow the polymer to be arranged in layers. In the *β* form of PAALA-1 and PAALA-2, the hydrogen-bonded layers are separated by a distance of 7.5 and 8.4 Å, respectively. These spacings correlate well with those reported for poly(α-L-aspartate)s and poly(α-L-glutamate)s[12] provided that differences in the number of methylene units contained in the side chain are taken into account. The unit height observed for the two polymers is 4.61 Å, indicating that exactly the same conformation is adopted in both cases. The shortening of the repeating unit with respect to which would be a fully extended conformation is just about 0.2 Å. This contraction is of an extent similar to that occurring in the pleated *β*-sheet of polypeptides.[12]

Unfortunately, the diffraction data available on the *β* form of PAALA-1 and PAALA-2 are clearly insufficient to ascertain the precise arrangement of the molecules in the unit cell. By analogy with the *β* structure described for poly(*γ*-methyl α-L-glutamate), a sound model enabling an efficient packing of the side chains in the interlayer space may be tentatively proposed for this crystal form. [30] A crude schematic representation of such a model is given in Figure 11. The hydrogen-bonded sheets are made of antiparallel chains so that the *a* repeat of the structure corresponds to twice the interchain distance. In order to account for the *b* dimension the successive layers are assumed to be sheared alternately up and down by ±1/4*c*. This arrangement is of the type described for the α form of nylon 6[31] and not far from the model put forward for the predominant crystal form of nylon 3,[32] where successive layers are shifted ±1/2*c*. A detailed analysis of this structure combining energy calculations and modeling simulations will be published in the near future.

It should be said that the distance between hydrogen-bonded chains estimated for the *β* structure is out of the 4.7–4.9 Å range commonly accepted for polypeptides and nylons.[33,34] Poly(L-lysine)[35] (4.62 Å) and the alternating copolypeptide of ethyl glutamate and glycine[12] (4.64 Å) are exceptional cases displaying interchain distances close to those reported here. A more suitable value for the hydrogen bond length would result if a slight deviation from orthogonality, not perceivable by X-ray diffraction, was assumed to occur in the structure. In such a case, a much poorer correlation between observed and calculated spacings would be attained. Nevertheless the occurrence of a monoclinic structure with a *γ* angle close to 90° cannot be completely discarded.

Concluding Remarks

Several remarkable conclusions about the conformation of poly(α-alkyl *β*-L-aspartate)s which contribute to outline a comprehensive picture of the structural behavior of this family of polyamides may be drawn from this work.

(a) Conformations of α-helix type are common to the whole family of poly(α-alkyl *β*-L-aspartate)s whatever the length of the alkyl side group. Extended conformations are exclusively adopted by the ethyl and methyl derivatives.

(b) The symmetry of the poly(*β*-L-aspartate) helix appears to be highly sensitive to the nature of the side chain. Although the 13/4 helix is by far the arrangement more frequently observed among poly(*β*-L-aspartate)s, helical conformations with 17/4 and 4/1 symmetries are also observed for members containing four or less carbon atoms in the alkyl side chain.

(c) Crystal polymorphism is a usual property of poly(α-alkyl *β*-L-aspartate)s and becomes more pronounced as the size of the alkyl group decreases. The maximum of conformational variability is observed for the ethyl derivative, which is able to adopt three different crystal forms.

(d) The structural diversity displayed by poly(β-L-aspartate)s entails a range of conformational parameter values wider than in the case of the related polypeptides poly(α-L-aspartate)s and poly(α-L-glutamate)s. The presence of one additional methylene in the backbone of the repeating unit of poly(β-L-aspartate)s is thought to be responsible for the higher chain flexibility displayed by these polyamides.

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