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Analysis of the Helical Conformations in Poly(β-L-aspartate)s: Poly(α-*n*-butyl β-L-aspartate) and Poly[α-(2-methoxyethyl) β-L-aspartate]

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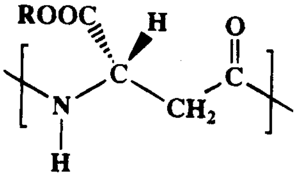
ABSTRACT: The conformation of the two title poly(β-L-aspartate)s was analyzed by both molecular mechanics calculations and the linked-atom least-squares (LALS) methodology using X-ray diffraction data previously published by us. A right-handed 13/4 helix composed of 14-member hydrogen-bonded rings was found to be the most favored conformation for the so-called "hexagonal form" in which both compounds are known to crystallize. The preferred conformation for the tetragonal form, which is the second crystal modification adopted by the *n*-butyl derivative, consisted of a right-handed 4/1 helix. Different modes of packing compatible with experimental data were examined for each crystal form, the most favored being those corresponding to space groups $P2_1$ and $P4_1$ for hexagonal and tetragonal, respectively. These results appear to be substantially identical to those earlier described for poly(α-isobutyl β-L-aspartate) which is the only poly(β-L-aspartate) investigated so far by these methods. Comparison between the relative stabilities calculated for the two polymorphs of each polymer allowed one to account for the different tendency to crystallize in the tetragonal form that is experimentally observed among poly(β-L-aspartate)s possessing side chains of intermediate size.

Introduction

A variety of synthetic polyamides, all of which having in common the ability to display helical conformations, has been investigated in our laboratory during the last decade. They may be classified into two groups. One group includes aliphatic polyamides and copolyamides containing isolated methylenes which tend to crystallize in 3-fold or 6-fold helical structures with hydrogen bonds intermolecularly arranged.[1-4] The other group consists of stereoregularly substituted nylons 3 and 4 taking up helical conformations stabilized by intramolecular hydrogen bonds with features similar to the familiar α-helix of polypeptides.[5-8] Within the latter, main efforts have been devoted to studying poly(α-alkyl β-L-aspartate)s, which are nylon 3 derivatives bearing an alkoxycarbonyl group attached to the backbone β-carbon of every repeating unit.



Poly(α-isobutyl β-L-aspartate), abbreviated PAIBLA, was the first poly(β-L-aspartate) for which an α-helix-like structure was reported.[5] Depending on the conditions used for the preparation of the sample,[6,7] two crystal forms, namely hexagonal and tetragonal, were found for this compound. Both forms have been extensively characterized and the conformation of the chain in each of them has been analyzed in full detail.[9,10] The so-called hexagonal form actually consists of an orthorhombic lattice composed of right-handed 13/4 helices arranged antiparallel. For simplicity, this form is usually described as a hexagonal pseudocell with parameters $a = b = 13.5$ Å and $c = 19.9$ Å. The tetragonal form is a parallel arrangement of right-handed 4/1 helices in a lattice of parameters $a = b = 13.5$ Å and $c = 4.94$ Å. The hexagonal form may be converted into tetragonal either by heating at temperatures above 200 °C or by contact with certain alcohols.[7] Some interesting properties closely related to the helical character of the macromolecule, such as formation of lyotropic mesophases[11] and piezoelectricity[12] have been reported for the hexagonal form of this polymer.

We have shown later that other poly(β-L-aspartate)s bearing alkyl side chains of different sizes and shapes behave similarly to PAIBLA.[13] It seems that the ability to adopt α-helix-like conformations is a feature common to the whole family of these polymers. The structure of two members of the family, namely poly(α-*n*-butyl β-L-aspartate) (PANBLA) and poly[α(2-methoxyethyl) β-L-aspartate] (PAMELA), respectively, has been recently examined in some detail by X-ray diffraction of fibers and films.[14,15] Two crystal forms with characteristics very close to the hexagonal and tetragonal forms of PAIBLA were found for PANBLA although the hexagonal–tetragonal transition taking place in the former by the effect of heating is not observable in the latter. A crystal structure substantially identical to the hexagonal form of PANBLA and PAIBLA was found to be adopted by PAMELA too. Nevertheless, this polymer is distinguished from the other two by not being able to crystallize in tetragonal form.

In the present work we have carried out a conformational analysis of PANBLA and PAMELA and results obtained thereof are critically compared with those reported on PAIBLA. The focus of this study is to evaluate the influence of the constitution of the side group on the conformation of these poly(β-L-aspartate)s and to explain the differences observed among them regarding the formation of tetragonal structures. Two well-established methodologies, energy calculations and linked-atom least-squares refinements (LALS), have been used. Energy calculations have been performed on an isolated polymer chain as well as on packing

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models compatible with the crystal structures presumed for the polymorphs. X-ray diffraction data on PANBLA and PAMELA previously reported by us were used for the LALS analysis, in which both packing and side chain geometrical restrictions were simultaneously considered. Results obtained by both methodologies appear to be consistent and well supporting to each other in spite of the scarcity of experimental data available for some of the examined structures.

Methods

Energy Calculations. These calculations were performed with explicit consideration of all atoms using the AMBER 3.0 Rev.A[16] program with all-atom parametrization.[17] A similar electrostatic charge distribution was considered for the backbone atoms of the three polymers. Parametrization of the side chain atoms of PANBLA and PAMELA was made following a methodology previously developed by us and published elsewhere.[18,19] Charge parameters reported for PAIBLA[9] were re-evaluated using this methodology in order to make them comparable in the context of this work. The charge values resulting for the monomeric unit of the three polymers are given in Figure 1. Parameters for the bonding terms of the ester side group previously used for PAIBLA[9] were adopted for the three cases.

Energy optimizations were carried out in two steps. In a first stage, the worst steric conflicts in the starting conformations were removed by 300 cycles of steepest descent optimization. Subsequently, the resulting conformations were optimized using a conjugate gradient algorithm until the difference in energy was less than 10^{-7} kcal/mol·residue or the norm of the gradient for two successive minimization steps was less than 0.1 kcal/mol·residue. In order to evaluate the influence of the polarity of the medium, calculations were made for two different expressions of the dielectric constant ($\epsilon = 1$ and $\epsilon = 1r$, r being distance dependent). 1−4 interactions were scaled to 0.5 and nonbonding interactions were cut off at 8 Å.

Approximate models with a sound stereochemistry were built for both 13/4 and 4/1 helices using standard bond lengths and angles. A first selection was made by molecular mechanics minimization of the conformational energy. Geometry optimizations were made at two levels of approximation. First, the conformation of an isolated chain of polymer was optimized. Second, the relative energies of different packing models suitable for the hexagonal and tetragonal crystal forms were calculated. In all cases, models were generated by considering a set of 12 chains, so that the crystal environment could be rigorously mimicked within the space confined to the cutoff distance. The packing energy was determined by taking into account the nonbonded interactions between atoms contained in the central monomeric units.

To figure out the minimum chain length required to avoid end effects on the central part of the molecule, chain segments with a number of residues varying from 6 to 24 were subjected to pilot calculations. Figure 2 shows the deviations of the dihedral angles φ, ξ, and ψ *vs* the residue number for energy-minimized 13/4 and 4/1 helices of PANBLA composed of 18 residues and blocked at the amino terminal end with an acetyl group and with an *N*-methylamido group at the carbonyl end. Such results indicate that a chain segment composed of 18 residues may be considered sufficiently accurate to simulating a polymer chain of infinite length.

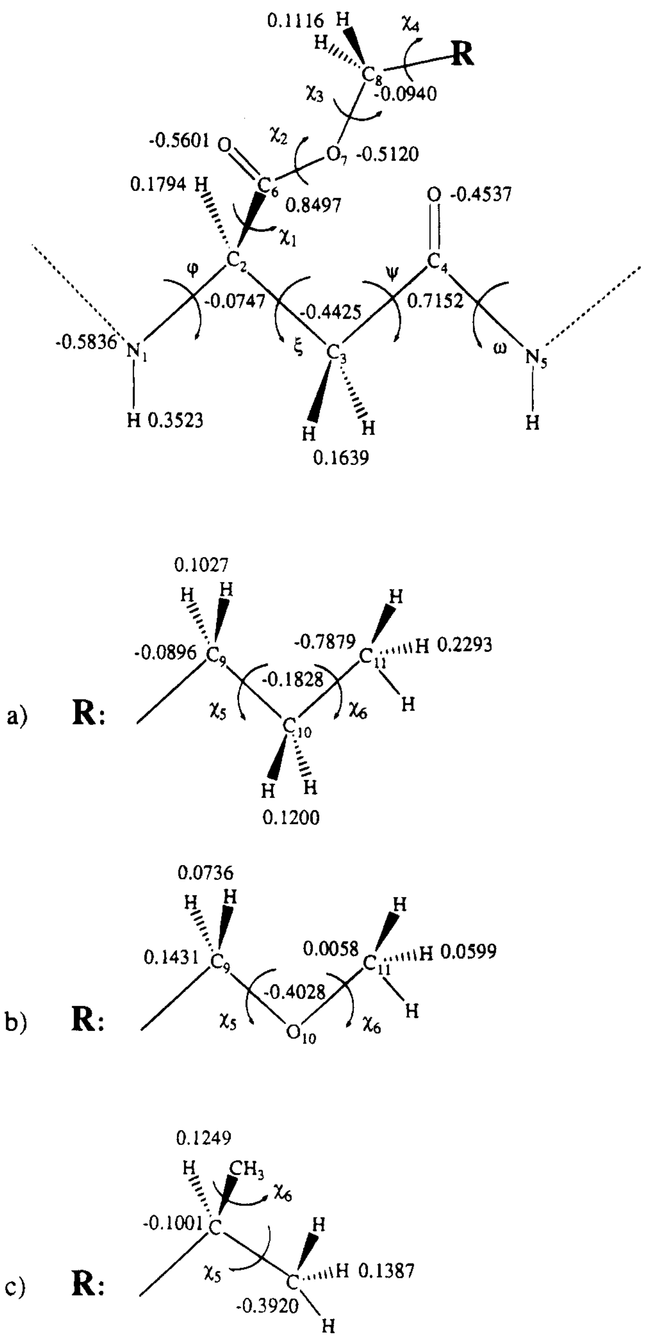

Figure 1. Electrostatic charges computed for (A) poly(α-*n*-butyl β-L-aspartate), (B) poly[α-(2-methoxyethyl) β-L-aspartate], and (C) poly(α-isobutyl β-L-aspartate). The torsion angles are indicated.

Structural Modeling by LALS. The averaged coordinates of helices optimized by energy calculations were used as the starting conformations for LALS refinements.[20] Standard bond lengths and angles were kept fixed and the torsion angle ω was held in the *trans* conformation. Hydrogen bonds were assumed to be almost linear with an approximate length of 2.90 Å. Firstly, the conformation of the main chain was refined against contour conditions, i.e., continuity of the helix and repeating unit length. Secondly, the crystal structure was refined against X-ray data taking into account the side-chain geometry so that intra and intermolecular contacts could be simultaneously minimized at this stage.

Fiber X-ray diffraction patterns such as those reported in refs 14 and 15 were used for the analysis of

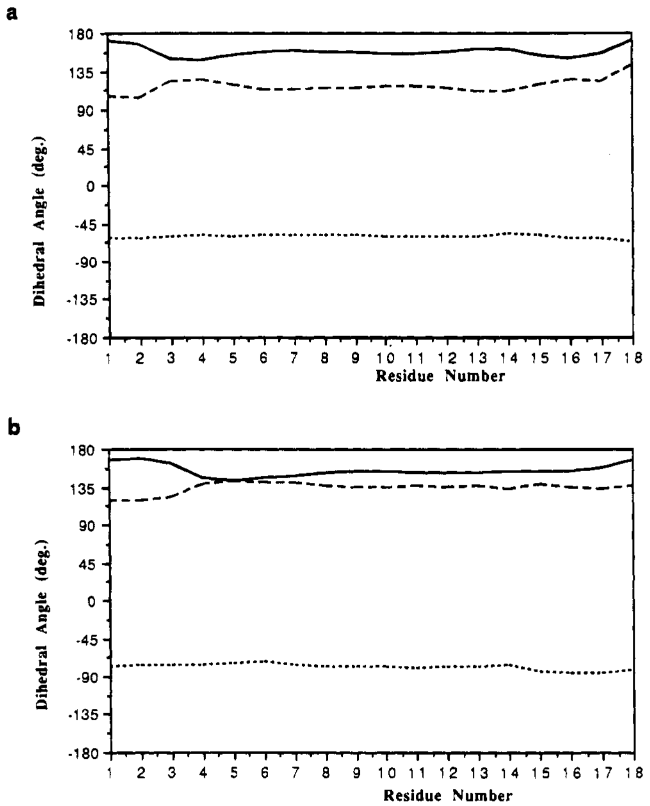

Figure 2. Deviations of the dihedral angles φ (—), ξ (- - -), and ψ (— — —) *vs* the residue number in a chain of 18 residues. (a) and (b) correspond to the 13/4 and 4/1 helices, respectively.

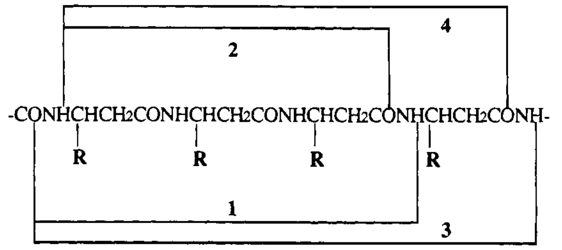

Figure 3. Different hydrogen-bonding schemes compatible with the helical conformations of a poly(β-L-aspartate) chain.

the crystal structure of the tetragonal form of PANBLA and the hexagonal form of PAMELA, respectively. The intensities were measured with a unidimensional Joyce-Loebl MK III CS microdensitometer. Radial scans were registered and corrections were made to take into account the orientation of the diffracting planes. The integrated areas under each spot were then corrected for polarization and Lorentz factors. No absorption correction was needed since the films used for diffraction were thin. Multiplicity corrections were introduced when required.

Calculations were performed on a Silicon Graphics station RI-4000 at our laboratory and on an IBM-3090/600 and a CRAY-YMP at the Centre de Supercomputació de Catalunya (CESCA).

Results

As noted in earlier works[6,9] and schematically indicated in Figure 3, at least four different models turn out to be stereochemically feasible for both the hexagonal and the tetragonal crystal forms of poly(β-L-aspartate)s. Hydrogen-bonding schemes **1** and **2** give rise to 13/4 helices suitable for the hexagonal structure whereas schemes **3** and **4** generate 4/1 helices compatible with the tetragonal one. In addition, each arrangement may be conceived in either right or left-handed conformation. A 4/1 helix with the amido groups alternately oriented in opposite directions, which is inspired in the so-called π-helix of polypeptides,[21] may be also considered suitable for the tetragonal form. All these possibilities were examined by energy calculations, and the resulting optimized conformations were used for building the crystal structures to be subjected to refinement by LALS.

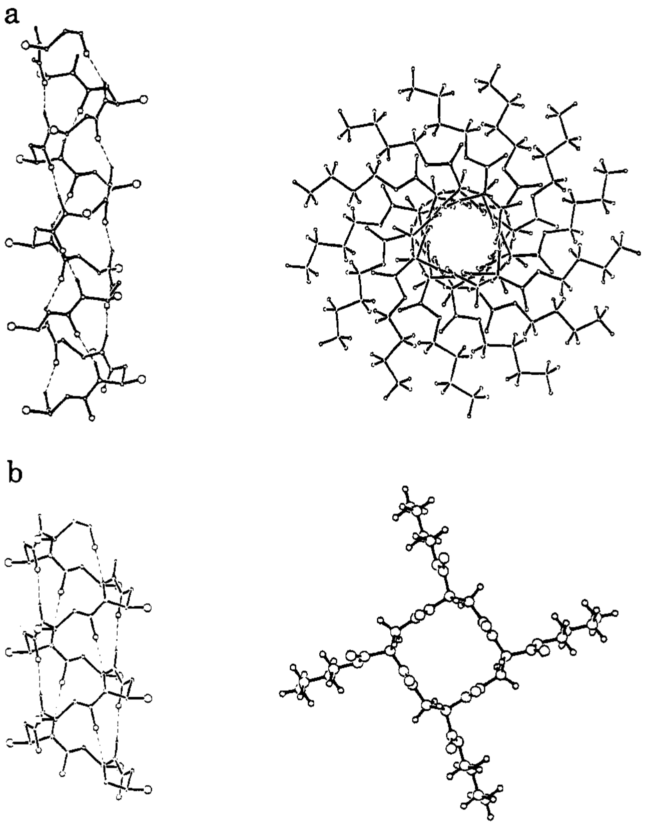

Figure 4. Axial and equatorial projections of the most favored models of poly(α-n-butyl β-L-aspartate) according to the hydrogen-bonding schemes **2R** (a) and **4R** (b). For clarity, the lateral side chains have been substituted by a single sphere in the axial projections.

Energy Calculations. Poly(α-*n*-butyl β-L-aspartate). Molecular mechanics calculations carried out on an isolated chain of polymer showed that the right-handed models **2R** and **4R** are the most stable conformations for the hexagonal and tetragonal forms of PANBLA, respectively. These optimized conformations are diagrammatically represented in Figure 4, and their averaged torsional angles are given in Table 1. The low standard deviations observed for the dihedrals in both models indicate that the conformation of the asymmetric unit is practically constant throughout the whole polymer chain. On the contrary, standard deviations up to 35–36° were found for the left-handed model **1L**, which tended to adopt a distorted conformation with subsequently losing the initial hydrogen bond scheme. With regards to models **1R** and **2L**, they were found to be sterically hindered and geometry optimizations invariably led to a disruption of the helical symmetry. This was also the result attained in the analysis of all the arrangements considered for the 4/1 helix except in the case of model **4R**, which turns out to be the only conformation sterically accessible to the tetragonal form. These results were essentially independent of the dielectric with only minor variations in the dihedrals

Table 1. Stereochemically Possible Conformations Obtained from the Energy Minimization of an Isolated Chain of Poly(α-*n*-butyl β-L-aspartate) and Poly[α-(2-methoxyethyl) β-L-aspartate] for $\epsilon = 1$

	torsion angles[b]										
model[a]	φ	ξ	ψ	ω	χ_1	χ_2	χ_3	χ_4	χ_5	χ_6	ΔE[c]
					Poly(α-*n*-butyl β-L-aspartate)						
2R	155.4 (4.1)	−60.5 (1.0)	116.7 (4.5)	−178.6 (0.9)	178.8 (2.4)	175.7 (2.9)	172.4 (1.6)	−175.0 (0.9)	−179.9 (0.7)	−179.3 (0.6)	0.0
4R	150.9 (5.2)	−78.4 (3.3)	135.4 (4.9)	174.6 (2.8)	170.4 (1.8)	177.9 (2.8)	177.7 (0.7)	−176.9 (2.0)	−178.7 (0.5)	−179.5 (0.5)	0.2
					Poly[α-(2-methoxyethyl) β-L-aspartate]						
2R	154.2 (5.2)	−59.2 (1.1)	117.5 (5.5)	−178.7 (1.3)	177.2 (2.5)	174.0 (3.2)	171.9 (2.0)	−176.7 (0.9)	177.9 (0.6)	−178.9 (0.2)	0.0
4R	148.2 (6.2)	−77.7 (3.3)	137.5 (6.2)	173.0 (2.7)	169.2 (1.7)	175.6 (3.0)	176.1 (1.2)	−179.9 (1.0)	172.0 (2.2)	178.5 (1.1)	0.8

[a] Models **2R** and **4R** refer to right-handed helices with the hydrogen-bonding schemes **2** and **4** shown in Figure 3. [b] Averaged backbone and side-chain conformational angles and standard deviations (in parentheses) for the 12 central residues. Units in degrees. [c] Relative energies of the models for the same polymer. Units in kcal/mol·residue.

being observed for different expressions of ϵ. Comparison of the relative energies calculated for models **2R** and **4R** reveals that 13/4 and 4/1 helices of PANBLA are almost isoenergetic with differences of 0.2 and 0.7 kcal/mol·residue in favor of the former for a dielectric constant of 1 and 1*r*, respectively. These energy differences are significantly smaller than those calculated for such polymorphs in the case of PAIBLA, where model **4R** was found to be more stable than **2R** by 5.7 kcal/mol·residue.

The packing of the helices in their corresponding crystal forms was then investigated by taking into account the influence of nonbonding interactions between side chains. Hexagonal and square arrays of **2R** and **4R** helices, respectively, were built in both parallel and antiparallel arrangements. The starting models used for calculations were generated by systematic rotation of one chain around its axis at intervals of 45° within the range 0−180° while the neighboring chains were held at their initial orientations. For the hexagonal form, the minimum energy was provided by an antiparallel arrangement of chains rotated by 180° to each other. This mode of packing corresponds in fact to an orthorhombic lattice with space group $P2_1$. Conversely, a parallel arrangement of helices, all of them with the same orientation around the chain axis, which corresponds to the space group $P4_1$, was found to be the most stable mode of packing for the tetragonal form.

The conformational parameters and relative energies calculated for the most favored packing models for each crystal form are given in Table 2. As can be seen, values for backbone torsion angles are very close to those obtained for the corresponding isolated chains, indicating that the conformations are barely sensitive to packing effects. This must be attributed to the great stability provided to the helices by intramolecular hydrogen bonding. On the contrary, significantly larger standard deviations are displayed by the dihedral angles of the side chains, in particular when the hexagonal form is concerned. This is due to the fact that conformations of all side-chain groups have been averaged in order to give a representative picture of the structure. Obviously, this is physically unreasonable for a 13/4 helical symmetry since the packing environment fluctuates, and in consequence the side chain should adopt a different conformation according to its position along the helix. A similar situation was found in the case of PAIBLA which has been interpreted in similar terms.[9]

Poly[α-(2-methoxyethyl) β-L-aspartate]. A calculation strategy similar to that described above in the analysis of PANBLA was followed in this case. Although the tetragonal crystal form has not been experimentally observed for this polymer, this structure was also subjected to modeling analysis and energy calculations for the sake of comparison. Molecular mechanics calculations on an isolated chain of polymer led us to conclude that models **2R** and **4R** are the most favored conformations for the 13/4 and 4/1 helices of PAMELA, respectively. Results obtained for these models are given in Table 1 which shows how all backbone dihedral angles turn out to be very similar to those obtained for PANBLA. However, the relative energies calculated for the two helices are reversed when compared with those displayed by either PAIBLA or PANBLA. In fact, the energy of model **2R** was found to be 0.8 kcal/mol·residue lower than that calculated for model **4R**. It is evident that intramolecular side-chain interactions in PAMELA must contribute to the destabilization of the 4/1 helix, as reasonably expected from the proximity attained by the polar side chains in this structure.

Minimizations of packing energies were carried out by starting from the same type of chain arrangements previously described for PANBLA, and the obtained results are shown in Table 2. The arrangements favored by these calculations turn out to be the same as those obtained for PAIBLA and PANBLA. In this case, however, it is the hexagonal structure which appears to be stabilized with respect to the tetragonal one by 2.0 kcal/mol·residue. This is in full agreement with the result obtained for an isolated chain and reveals that intermolecular side-chain interactions additionally contribute in a significant extent to the destabilization of the tetragonal form.

LALS Calculations. The minimum energy conformations obtained in the preceding section were used for building the models of PANBLA and PAMELA to be analyzed by LALS. Backbone and side-chain dihedral angles were first refined under the constraints required to preserve both the helix continuity and the hydrogen-bonding scheme. Subsequently, the helical geometry was refined by introducing packing constraints for the experimentally determined crystal parameters and lattice symmetry. Space groups $P2_1$ and $P4_1$ derived from energy calculations were considered for the hexagonal and tetragonal crystal forms, respectively. As logically expected, no appreciable changes in the dihedral angles of the refined models were observed with respect to those obtained from molecular mechanics calculations. Finally, the packing of the chains in the tetragonal model of PANBLA and in the hexagonal model of PAMELA was refined against the intensities observed in their respective fiber X-ray diagrams. The hexagonal form of PANBLA could not be refined in this way due to the poor quality of the diffraction data available for this polymorph. Dihedral angles, crystal parameters, and hydrogen-bonding geometries for the refined crystal models of PANBLA and PAMELA are listed in Table 3, and the atomic coordinates of the residue in each model are given in Table 4.

Table 2. Results Obtained from Energy Minimization of the Most Favored Modes of Packing for the Hexagonal and Tetragonal Crystal Forms of Poly(α-*n*-butyl β-L-aspartate) and Poly[α-(2-methoxyethyl) β-L-aspartate]

space group[a]	torsional angles[b]										ΔE[c]
	φ	ξ	ψ	ω	χ_1	χ_2	χ_3	χ_4	χ_5	χ_6	
					Poly(α-*n*-butyl β-L-aspartate)						
$P2_1$	152.5 (11.7)	−64.2 (6.4)	122.9 (14.6)	−174.9 (7.9)	−170.9 (23.0)	166.2 (21.2)	176.6 (7.2)	−179.2 (10.3)	173.2 (17.1)	−171.2 (42.2)	0.6
$P4_1$	148.4 (6.6)	−76.4 (6.6)	138.2 (7.1)	171.9 (2.2)	163.0 (8.2)	−166.9 (19.2)	−169.9 (14.8)	−176.4 (8.8)	−176.4 (6.4)	−175.0 (3.9)	0.0
					Poly[α-(2-methoxyethyl) β-L-aspartate]						
$P2_1$	151.5 (12.5)	−58.2 (11.7)	120.7 (16.0)	−177.4 (5.6)	−162.7 (37.5)	165.6 (27.2)	176.5 (52.7)	−166.0 (39.0)	169.8 (33.0)	178.1 (36.9)	0.0
$P4_1$	145.7 (4.2)	−70.2 (2.9)	138.1 (5.3)	166.2 (1.9)	159.8 (4.7)	−152.1 (3.4)	166.0 (4.7)	−167.0 (4.1)	−179.4 (1.4)	−162.8 (1.8)	2.0

[a] Space group $P4_1$ corresponds to the tetragonal form, whereas space group $P2_1$ corresponds to the hexagonal form (see text). [b] Averaged backbone and side-chain conformational angles and standard deviations (in parentheses) for the 16 central residues. Units in degrees. [c] Relative energies with respect the most favorable mode of packing. Units in kcal/mol·residue.

Table 3. Conformational Parameters, Hydrogen Bond Geometries, and *R* Factors Obtained from LALS Refinements for the Crystal Forms of Poly(α-*n*-butyl β-L-aspartate) and Poly[α-(2-methoxyethyl) β-L-aspartate]

	PANBLA		PAMELA
	pseudo-hexagonal[a]	tetragonal	pseudo-hexagonal[b]
helix symmetry	13/4	4/1	13/4
space group	$P2_1$	$P4_1$	$P2_1$
cell parameters			
a (Å)	13.45	14.15	12.40
b (Å)	13.45	14.15	12.40
c (Å)	20.60	4.90	20.74
α (deg)	90	90	90
β (deg)	90	90	90
γ (deg)	120	90	120
torsional angles (deg)			
φ	146.2	144.4	145.4
ξ	−59.8	−87.2	−59.6
ψ	128.8	148.1	130.0
ω	180.0	180.0	180.0
χ_1	173.4	146.4	168.9
χ_2	180.0	180.0	180.0
χ_3	180.0	180.0	180.0
χ_4	180.0	180.0	180.0
χ_5	180.0	180.0	180.0
χ_6	180.0	180.0	180.0
H-bond geometry			
d(H···O) (Å)	1.93	1.81	1.94
d(N···O) (Å)	2.88	2.82	2.90
∠NHO (deg)	172.3	178.2	173.1
R factor (%)	not calcd	15.6	12.9

[a] Cell parameters for the orthorhombic unit cell: a = 13.45 Å, b = 23.31 Å, c = 20.60 Å. [b] Cell parameters for the orthorhombic unit cell: a = 12.40 Å, b = 21.49 Å, c = 20.74 Å.

Twelve independent spots were used for refining the tetragonal form of PANBLA (Table 5). Comparison between the calculated and observed structure factors shows an overall good agreement, as reflected in a resulting R factor of 15.6%. The final conformational parameters for the helix in the model **4R** of PANBLA are φ = 144.4°, ζ = 87.2°, and ψ = 148.1°, which are substantially the same as those reported for the tetragonal form of PAIBLA.[9] A projection of the $P4_1$ unit cell down the chain axis demonstrating the interaction among side chains is displayed in Figure 5. In a manner similar to that which happens in the tetragonal form of PAIBLA, the helices are packed in parallel, which is a rather striking result. Nevertheless, the analysis of the antiparallel model for this form, which corresponds to the $P4_12_12$ space group, resulted in an R factor greater than 30%. This led us to reject the possibility of a packing with chains arranged antiparallel and confirmed the conclusions drawn by energy calculations.

Models **2R** of PANBLA and PAMELA were refined on the basis of the orthorhombic $P2_1$ space group implying an antiparallel arrangement of chains. Final values of the varied conformational angles are φ = 146.2°, ζ = 59.8°, and ψ = 128.8° for PANBLA and φ = 145.4°, ζ = 59.6°, and ψ = 130.0° for PAMELA. The small number of spots available made it advisable to consider all side chains with the same conformation, which is a rough approximation for a helix having 13/4 symmetry, as was revealed by energy calculations. In spite of this, no bad contacts were found and a satisfactory R factor of 12.9% was obtained for PAMELA, giving support to the feasibility of such a model. A molecular drawing of the unit cell of the hexagonal form made of **2R** helices projected down the helix axis is given in Figure 6, showing the essential features that were discussed in the Energy Calculations section.

Table 4. Cartesian Coordinates of One Residue for the Two Crystal Forms of Poly(α-*n*-butyl *β*-L-aspartate) and the Hexagonal Form of Poly[α-(2-methoxyethyl) *β*-L-aspartate]

	PANBLA						PAMELA		
	pseudohexagonal			tetragonal			pseudohexagonal		
atom	*x*	*y*	*z*	*x*	*y*	*z*	*x*	*y*	*z*
N1	0.7921	−1.9594	−0.2309	2.5589	−0.9958	0.0303	−0.8768	1.7975	1.5923
H−N1	0.4796	−2.1232	0.7155	2.4222	−1.1822	1.0135	−1.3712	1.5564	2.4394
C2	2.2126	−1.7052	−0.3718	3.3262	0.1982	−0.2662	−1.5674	1.4607	0.3626
H−C2	2.4260	−1.3881	−1.3926	3.0701	0.5586	−1.2734	−0.8336	1.3274	−0.4459
C3	2.6361	−0.5958	0.6087	2.9995	1.2836	0.7615	−2.3525	0.1619	0.5571
H1−C3	2.4296	−0.9149	1.6303	2.7854	0.8160	1.7339	−3.0622	0.2829	1.3888
H2−C3	3.7027	−0.4003	0.4983	3.8583	1.9634	0.8633	−2.9050	−0.0746	−0.3642
C4	1.8853	0.6730	0.3073	1.7836	2.0701	0.2996	−1.3894	−0.9704	0.8744
O=C4	1.5319	0.9616	−0.8438	1.5562	2.2438	−0.8966	−0.3881	−1.1643	0.1869
C6	2.9782	−2.9589	−0.0681	4.7908	−0.1204	−0.2074	−2.5138	2.5667	0.0007
O=C6	4.1621	−3.1005	−0.2034	5.6389	0.3784	−0.8944	−3.1654	2.6227	−1.0054
O7	2.1665	−3.9252	0.3825	5.0477	−1.0490	0.7240	−2.5475	3.5072	0.9546
C8	2.7898	−5.1921	0.7125	6.4343	−1.4441	0.8784	−3.4292	4.6344	0.7211
H1−C8	3.3318	−5.5680	−0.1552	6.8055	−1.8596	−0.0701	−3.1265	5.1526	−0.2008
H2−C8	3.4841	−5.0511	1.5409	7.0375	−0.5668	1.1548	−4.4639	4.2757	0.6165
C9	1.7059	−6.2077	1.1193	6.5440	−2.5041	1.9762	−3.3430	5.6035	1.9018
H1−C9	1.1811	−5.8454	2.0032	6.1736	−2.0894	2.9253	−3.6462	5.0864	2.8242
H2−C9	0.9965	−6.3301	0.3009	5.9416	−3.3822	1.7004	−2.3088	5.9633	2.0068
C10	2.3644	−7.5638	1.4340	8.0071	−2.9211	2.1391			
O10							−4.2734	6.7928	1.6554
H1−C10	2.8890	−7.9274	0.5504	8.3783	−3.3365	1.1907			
H2−C10	3.0736	−7.4426	2.2528	8.6103	−2.0438	2.4156			
C11	1.2806	−8.5794	1.8408	8.1168	−3.9811	3.2370	−4.1872	7.7620	2.8362
H1−C11	1.7466	−9.5393	2.0635	9.1687	−4.2809	3.3541	−4.8562	8.6170	2.6590
H2−C11	0.5711	−8.7018	1.0223	7.5144	−4.8591	2.9612	−3.1530	8.1217	2.9412
H3−C11	0.7577	−8.2171	2.7247	7.7464	−3.5664	4.1861	−4.4904	7.2449	3.7585

Table 5. Comparison of Observed and Calculated Spacings (*d*, in Å) and Structure Factors (*F*) for the Tetragonal Form of Poly(α-*n*-butyl *β*-L-aspartate)

spot	*d*(obs)	F_o	*hkl*[a]	*m*	*d*(calc)	F_c	diff
			Equatorial				
1	14.1	103.8	100	2	14.15	87.2	16.6
2	10.06	52.7	110	1	10.01	46.9	5.8
3	6.37	26.1	210	2	6.33	18.1	8.1
4	5.02	97.9	220	1	5.00	87.7	10.2
5	3.93	47.7	320	2	3.92	37.9	9.8
6	3.50	80.0	040	2	3.54	69.3	10.7
7	3.09	41.1	420	2	3.16	34.3	6.8
8	2.79	16.7	510	2	2.78	6.0	10.8
			1° Layer Line				
6	4.70	90.9	101	2	4.70	77.0	13.9
7	4.47	83.0	111	1	4.47	79.9	3.1
			2° Layer Line				
11	2.43	15.5	102	2	2.41	4.3	15.5
12	2.33	27.1	202	2	2.33	23.5	3.6

[a] Indexed on the basis of the tetragonal unit cell with $a = b = 14.15$ Å and $c = 4.90$ Å.

Table 6. Comparison of Observed and Calculated Spacings (*d*, in Å) and Structure Factors (*F*) for the Pseudohexagonal Form of Poly[α-(2-methoxyethyl) *β*-L-aspartate]

spot	*d*(obs)	F_o	*hkl*[a]	*m*	*d*(calc)	F_c	diff
			Equatorial				
1	10.8	124.4	110	2	10.74	141.4	−17.0
			020	1			
2	5.37	197.2	040	1	5.37	192.5	4.8
			220	2			
3	4.06	356.5	240	2	4.06	343.8	12.7
			310	2			
			150	2			
4	3.60	352.6	060	1	3.58	329.6	23.0
			330	2			
5	3.10	33.9	260	2	3.10	48.2	−14.3
			400	1			
6	2.99	180.4	170	2	2.98	155.9	24.4
			350	2			
7	2.70	119.5	080	1	2.68	107.8	11.7
			440	2			
			1° Layer Line				
8	9.70	59.3	021	1	9.54	66.2	7.1
			111	2			
9	6.02	151.3	201	1	5.94	123.0	28.3
			131	2			
10	5.30	264.5	041	1	5.20	278.5	−14.0
			221	2			
			4° Layer Line				
11	4.57	272.7	024	1	4.67	318.5	−45.8
			114	2			
			5° Layer Line				
12	3.88	254.2	025	1	3.87	268.0	−13.8
			115	2			
			8° Layer Line				
13	2.55	53.9	108	2	2.52	76.4	−22.5
			118	1			

[a] Indexed on the basis of the orthorhombic unit cell with $a = 12.40$ Å, $b = 21.49$ Å, and $c = 20.74$ Å.

Discussion

The two poly(*β*-L-aspartate)s analyzed in this study possess side chains of similar sizes but with constitutions different enough to make them distinct in their structural behavior. This may be applied also to PAIBLA, which is an isomer of PANBLA containing a branched butyl side group. While the same hexagonal structure is adopted by all three polymers under similar experimental conditions, the tendency to crystallize in tetragonal form follows the sequence PAIBLA > PANBLA ≫ PAMELA so that such a crystal form is not observable in the last case. The modeling analysis performed in this work was intended in part to rationalize this trend by comparing the conformational preferences of the three polymers in their respective crystal forms.

An antiparallel packing of 13/4 helices with a **2R** scheme of hydrogen bonds was found to be the model most favored for the hexagonal form of both PANBLA and PAMELA, which is indeed the model reported for the hexagonal form of PAIBLA too. Not only was this

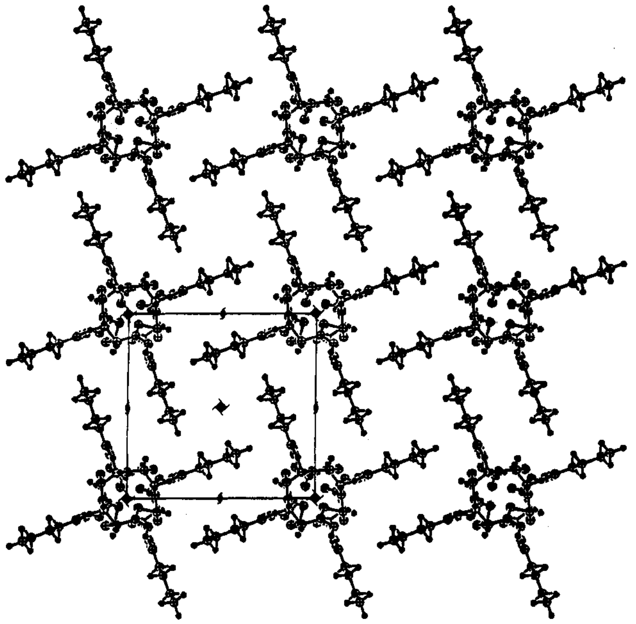

Figure 5. Projection along the c axis of the unit cell of the tetragonal form of poly(α-n-butyl β-L-aspartate) obtained from both energy and LALS calculations.

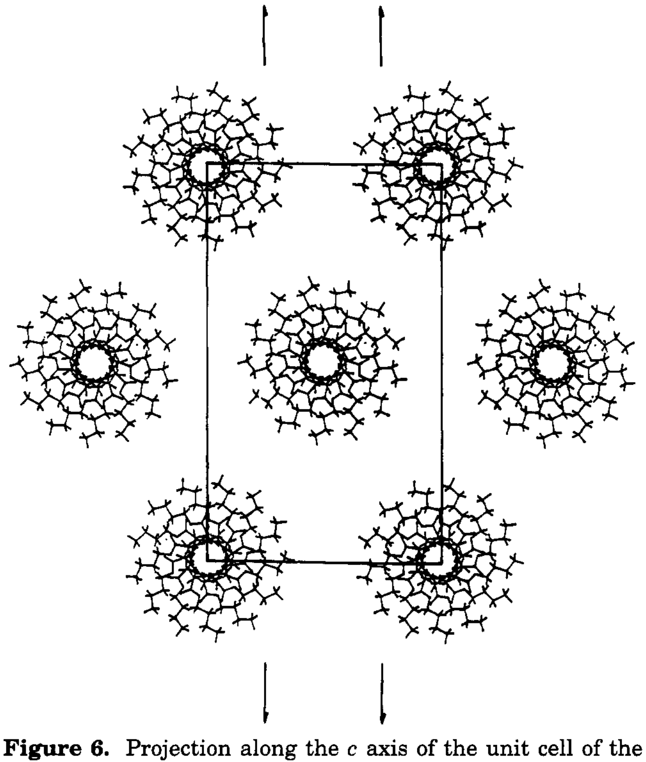

Figure 6. Projection along the c axis of the unit cell of the hexagonal form of poly(α-n-butyl β-L-aspartate) obtained from both energy and LALS calculations. The chains have been drawn with an increased separation between them in order to show the molecular arrangement more clearly.

model the most stable from an energetic point of view but it also gave a satisfactory R factor after refinement against X-ray data. Certain basic features of model **2R** are noteworthy. The right-handedness of the helix is contrary to expectations since the β-L-aspartic unit used as building block results in a repeating unit of D-amino acid-like configuration, for which a left-handed helix is usually preferred. On the other hand, hydrogen bonds and amido groups are oriented in an opposite sense, giving rise to a chain topology markedly different from that found in the α-helix of polypeptides. This feature has been recently investigated by polarized infrared spectroscopy which showed it to be fully consistent with the dichroic character displayed by the infrared spectrum produced by a right-handed 13/4 helix arranged according to scheme **2**.[22]

The packing of the side chains in the hexagonal structure is certainly peculiar. It should be emphasized that a static picture is given by molecular mechanics calculations for each side chain depending upon its axial position along the helix. All these conformations have been averaged in order to give a comprehensible picture of the whole structure. Nevertheless, the averaged conformation turns out to be almost extended, indicating that the side chains can be envisaged as protruding radially from the backbone. At packing, side chains from neighboring helices interpenetrate approximately three linkages for PANBLA and PAMELA, respectively, in agreement with the interchain distances observed for these two polymers. This mode of interaction fits well in the overall trend observed along the whole series of poly(β-L-aspartate)s containing linear alkyl side chains. Whereas the hexagonal form of methyl and ethyl poly(β-L-aspartate)s consists of independent helices with almost nontouching side chains, those members with alkyl groups composed of eight or more atoms organize in a layered structure with side chains segregated in an interlayer paraffinic phase.[23]

Regarding the tetragonal form, results from both energy calculations and LALS refinements are in favor of a parallel arrangement of 4/1 helices according to model **4R**. In this case, side chains are in an almost extended conformation too, but at difference with the 13/4 helix, torsional angles become affected by very small standard deviations, indicating that all side chains must have the same conformation, according to what is expected from the symmetry of the structure. The optimum packing of side chains is achieved at the expense of creating a void at the center of the unit cell. Since the existence of such a void is unfavorable from an entropic point of view, it may be reasonably anticipated that the tetragonal form should not be formed beyond a certain critical length. In fact, the experimental observation of this form in poly(β-L-aspartate)s bearing linear side chains is restricted to members with alkyl groups containing four chain atoms at maximum.

The conformational backbone angles calculated for the 4/1 helix of PANBLA and PAMELA are very similar and comparable to those described for the tetragonal form of PAIBLA. Therefore, the different tendency displayed by these three polymers toward the formation of the tetragonal form must arise from interaction effects mainly operating at the packing of the side chains in the solid state. A comparison of the overall relative energies calculated for the two forms of each polymer appears to be sufficient to account for the observed differences. However, in order to understand better the nature of the factors being mainly responsible for the destabilization of the tetragonal form, a comparison of the energies arising from the different types of interactions contributing to the total energy of the structure will be helpful. The contributions of the four energy terms computed for the hexagonal and tetragonal models of PAIBLA, PANBLA, and PAMELA are given in Table 7, which reveals that the relative stabilities of the two crystal forms are mainly determined by van der Waals and electrostatic interactions. The contribution of the van der Waals term is clearly in favor of the tetragonal form in both PANBLA and PAMELA whereas

Table 7. Energy Contributions[a] (in kcal/mol·residue) Computed for the Most Favored Models of the Poly(β-L-aspartate)s Studied in This Work

	space group	E_{bonded}	E_{vdw}	E_{elec}	E_{12-10}	E_{TOT}	ΔE^b
PAIBLA	$P4_1$	7.7	−4.9	−67.2	−0.5	−64.9	0.0
	$P2_1$	8.2	−5.4	−65.8	−0.2	−63.2	1.7
PANBLA	$P4_1$	5.7	−6.3	−45.2	−0.5	−46.3	0.0
	$P2_1$	5.7	−4.1	−46.8	−0.5	−45.7	0.6
PAMELA	$P4_1$	9.2	−7.9	−58.8	−0.5	−58.0	2.0
	$P2_1$	8.2	−5.6	−62.2	−0.4	−60.0	0.0

[a] The different energy contributions: E_{bonded} = bonding energy; E_{vdw} = van der Waals energy; E_{elec} = electrostatic energy; E_{12-10} = energy corresponding to the $r^{-12} - r^{-10}$ term; E_{TOT} = total energy. [b] Relative energy.

the reverse is observed to occur in PAIBLA. This is interpreted as a consequence of the more comfortable packing that may be achieved in the tetragonal form where side chains are linear. However, this effect is exceedingly outweighed by the electrostatic term operating in the opposite sense due to the strong side-chain interactions that take place under such conditions. The effect appears to be particularly pronounced in PAMELA where the intense repulsion between the highly charged oxygens results in a destabilization of the tetragonal form by 3.4 kcal/mol·residue with respect to the hexagonal one. The determining influence of the electrostatic interaction on conformation is even reflected in the isolated chain due to the 4/1 symmetry of the helix; the repulsive interaction between side chains of residues i and $i + 3$ increases the potential energy of the helix by about 0.8 kcal/mol·residue.

The results reported in this work on PANBLA and PAMELA along with data previously reported for PAIBLA allow us to approach the understanding of the structural preferences displayed by poly(β-L-aspartate)s bearing side chains of intermediate size. It has become clear that the stability of the tetragonal form in these poly(β-L-aspartate)s is determined by side-chain interactions and that the frequency with which such form is experimentally observed for each polymer correlates well with its stability relative to the hexagonal form. Nevertheless, the fact that the hexagonal−tetragonal transition taking place in PAIBLA by heating cannot be observed in PANBLA whereas the conversion is readily induced in both polymers by the effect of alcohols remains to be disclosed. This most likely has to do with the activation energy of the mechanism implied in the rearrangement of the structure, and it should be addressed by dynamic calculations.

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