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Molecular packing and conformational behaviour of poly(β-L-aspartate)s containing medium size linear alkyl side-chain

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The structure in the solid state of two helical nylon 3 derivatives, poly(α-*n*-hexyl-β-L-aspartate) and poly(α-*n*-octyl-β-L-aspartate), was investigated by molecular mechanics simulation methods. It was found that both polymers adopt the 13/4 helix previously observed for poly(β-L-aspartate)s bearing alkyl side chains of small size. The hexyl derivative could not form stable tridimensional structures. This is in agreement with reported experimental results which showed such polymers to be uncrystallizable. In the case of the octyl derivative, a crystal structure arranged in layers with a rhombic unit cell in the $P2_12_12$ space group appeared to be energetically favoured. Side chain trajectories within the interhelical space were found to vary depending on the position of the residue along the repeating unit of the helix. Side chains protruding normal to the sheets are almost extended whereas those located along the sheets are folded. A quantitative estimation of the penetration of the side chains into the intermolecular space has been made on the basis of interatomic distances measurements. © 1997 Elsevier Science Ltd.

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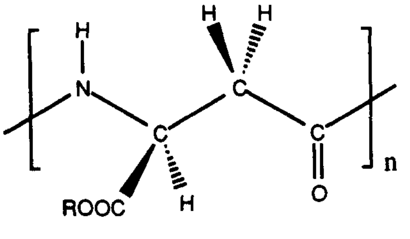
INTRODUCTION

Recently we have investigated the crystal structure of some poly(α-alkyl-β-L-aspartate)s, i.e. isobutyl, *n*-butyl and 2-methoxyethyl esters[1–7]. These polymers are stereoregular nylon 3 derivatives bearing an alkoxycarbonyl group attached to the backbone β-carbon of the repeating unit.



All these polymers are reported to adopt helical conformations similar to the α-helix typical of poly(α-amino acid)s. The most frequent crystal structure observed in this family of compounds consists of an monoclinic lattic composed of right-handed 13/4 helices arranged in antiparallel. For simplicity this form is usually described in terms of a hexagonal pseudocell containing only one chain. Both energy calculations and linked-atom-least-squares (LALS) refinements have shown that the poly(β-L-aspartate) 13/4 helix is right-handed and is stabilized by intramolecular hydrogen bonds set between the i and $i+3$ residues.

We have also investigated poly(β-L-aspartate)s bearing long linear alkyl side chains, abbreviated PAALA-n[8,9], n being the number of carbon atoms contained in the alkyl group. Compounds with $n = 6, 8, 12, 18$ and 22 were synthesized and their structure in the solid state examined by i.r. dichroism, n.m.r. and X-ray diffraction. Although all of them adopt the 13/4 helical conformation, the degree of ordering reached in the packing of the helices is dependent on the length of the alkyl side group. Biphasic structures with main chains arranged side-by-side in layers and side chains segregated in a paraffinic phase filling the interlayer space have been observed in PAALA-n, with $n \geq 12$[9]. This behaviour had been previously described for poly(γ-alkyl-α-L-glutamate)s containing more than ten carbons in the side chain[10]. PAALA-6 and PAALA-8 deviate from such behaviour because the alkyl side chains are too short to crystallize in a separate phase. As a consequence these polymers tend to adopt partially ordered structures with helices aligned along the molecular axis and side chains remaining in the molten state. PAALA-8 however is able to crystallize by annealing into a well-ordered tridimensional structure similar to those reported for poly(β-L-aspartate)s bearing short alkyl side chains[1,5,6]. In this case however the helices are not packed in a hexagonal array but in a layered structure with a basic unit cell of parameters $a = 18.0$ Å, $b = 12.3$ Å and $c = 19.9$ Å.

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Classical methods like LALS[11] are not suitable for studying the molecular structure of PAALA-6 and PAALA-8 at the atomic level because of the large number of rotational degrees of freedom present in these compounds. Methods based on energy calculations have proven to be also reliable in the conformational study of poly(β-L-aspartate)s and they are not restricted by molecular size so severely as the former[3,4,6]. In this work we present a computational study of the structure of both PAALA-6 and PAALA-8 by molecular mechanics methods with the purpose of disclosing what is the precise molecular arrangement adopted by these polymers in the crystalline state. Results from this study will be of use as the starting point for modelling the biphasic structure characteristic of PAALA-*n* with $n = 12, 18$ and 22. These polymers are of particular interest because they form liquid-crystal phases with striking thermochromic properties.

METHODS

Computational details

Calculations were performed using the AMBER 3.0 Rev.A program[12] with all-atom parametrization[13]. The electrostatic charge distribution for the backbone atoms was the same as that we obtained previously for other poly(β-L-aspartate)s[6]. Parametrization of the side chain atoms of PAALA-6 and PAALA-8 was made following the same procedure used in previous works[3–6]. The charge values resulting for the monomeric units of such polymers are given in *Figure 1*. Parameters for the bonding terms of the ester group previously developed for the poly(α-isobutyl-β-L-aspartate)[3] were adopted in this case, whereas the remainder parameters were taken from the AMBER libraries[12,13].

Geometry optimizations were carried out in two steps. First the worst steric conflicts were removed by 300 cycles of steepest descent optimization. Then the resulting conformations were optimized using a conjugate gradient algorithm. 1–4 interactions were scaled to 0.5 and nonbonding interactions were cut-off at 6.5 Å. The list of nonbonded pairs was updated every 25 cycles. Calculations were performed on a Silicon Graphics station RI-4000 at our laboratory and on a CRAY-YMP at the Centre de Supercomputació de Catalunya (CESCA).

Construction of the simulated models

The 13/4 helical conformations of PAALA-6 and PAALA-8 were constructed according to ref. 6. The conformation of such polymers corresponds to a right-handed 13/4-helix with $c = 19.9$ Å. Each helix was composed of 18 residues and blocked at the amino terminal end with an acetyl group and with an

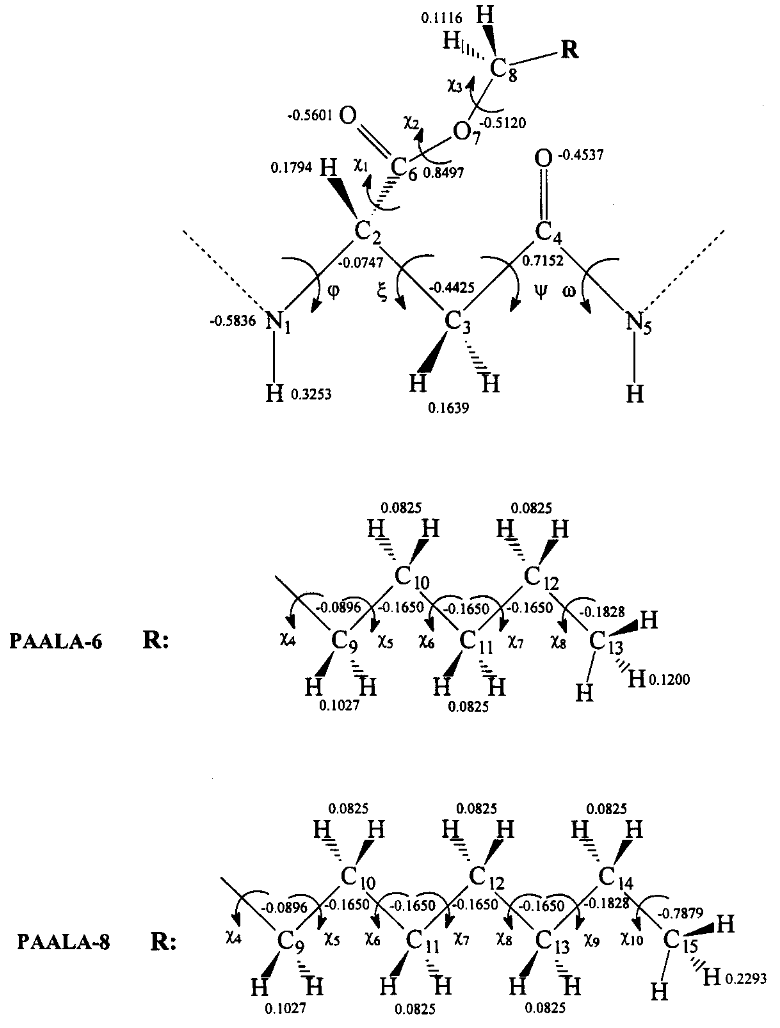

Figure 1 Electrostatic charges computed for PAALA-6 and PAALA-8. The torsion angles are indicated

N-methylamido group at the carbonyl end. Previous results indicated that a chain segment composed of 18 residues is sufficiently accurate to simulate a polymer chain of infinite length[6]. The backbone torsional angles ϕ, ξ and ψ (see *Figure 1*) were obtained from previous LALS refinements of other poly(β-L-aspartate)s. An extended conformation was initially considered for all the alkyl side groups since this is the lowest energy arrangement found for the two polymers when an isolated molecule is considered.

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 cut-off distance. According to previous results on poly(β-L-aspartate)s[3,6], the two chains in the unit cell were placed in antiparallel arrangement. By this means a lattice with space group $P2_1$ was built for PAALA-6, whereas for PAALA-8 both $P2_1$ and $P2_12_12$ space groups were taken into account. The initial separation between helices along the *a*- and *b*-axes was imposed to be 6 Å greater than the experimentally estimated intermolecular distances. At these exceedingly large distances the helix–helix interactions are almost negligible and unfavourable steric interactions are avoided. The systems were then relaxed keeping both the backbone torsional angles and the lattice parameters constrained to their initial values. The constraining force constants were 500 kcal mol^{-1} rad^{-2} and 1000 kcal mol^{-1} Å^{-2} for angles and distances respectively. In order to generate a reasonable molecular model of the crystal the same computational strategy was applied to the two polymers. First, the lattice was compressed 0.3 Å along *a*- and *b*-directions simultaneously and the system optimized under the same constraints. The process was repeated using in each cycle the minimized coordinates resulting in the previous step until the experimental dimensions of the lattice were reached. The final optimization of the structure was performed in two stages. First, the constraints of the backbone torsional angles were removed and the energy of the system minimized

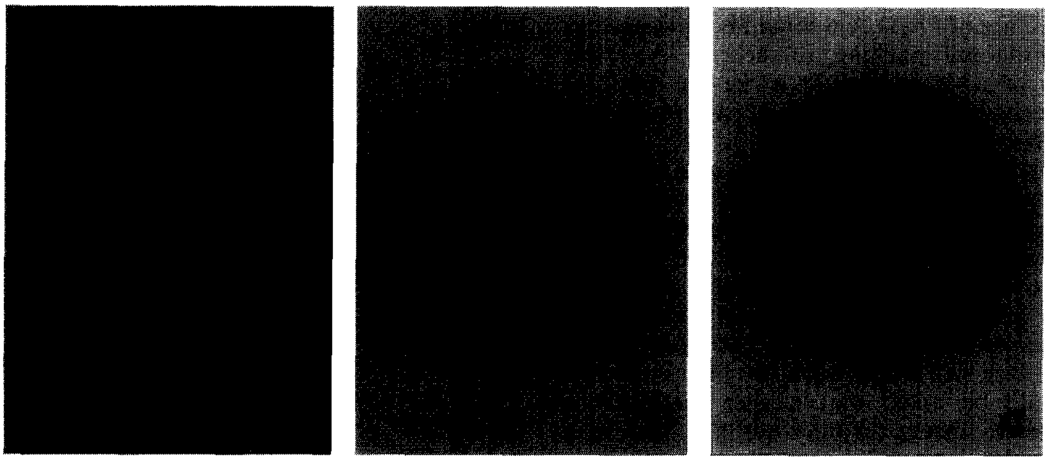

Figure 2 X-ray diagrams of stretched films of PAALA-6 (a) and PAALA-8 (b). (c) Diagram of PAALA-8 crystallized by annealing

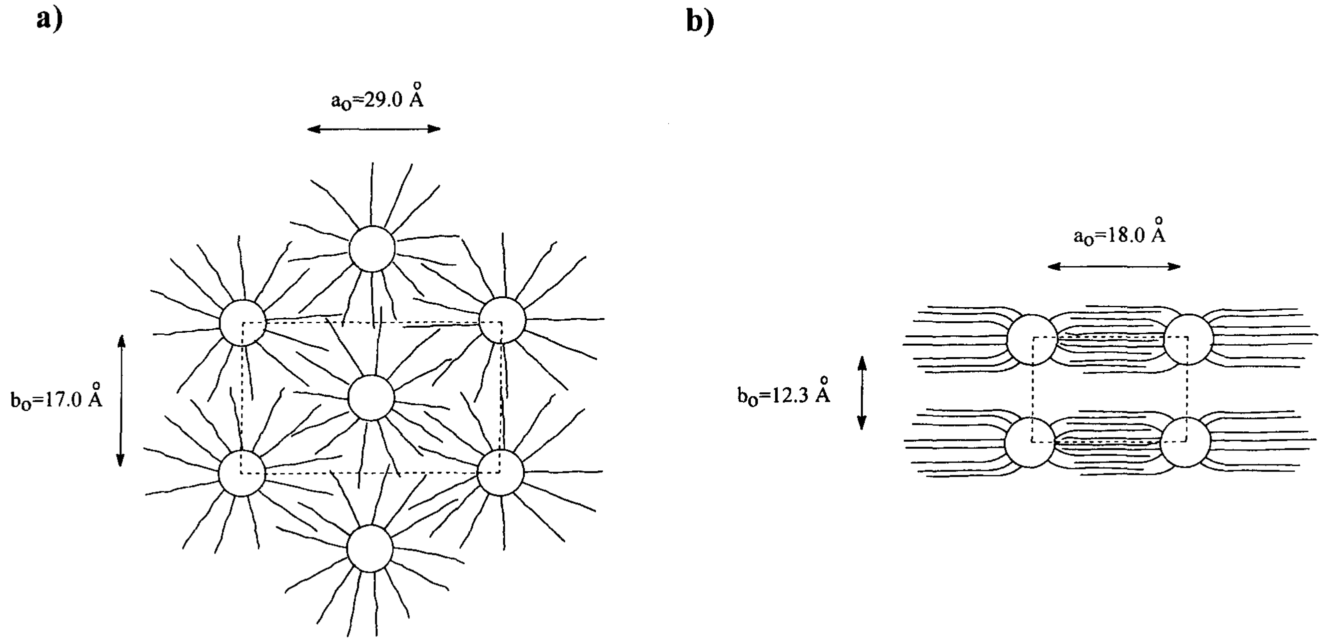

Figure 3 Schematic picture of the hexagonal structure of PAALA-6 (a) and the rectangular lattice form of PAALA-8 (b). The drawing is not scaled

(named stage **A** in the text). Second, a geometry optimization of the whole system was performed without applying any constraint (named stage **B** in the text). It should be noted that the molecular system used for studying PAALA-6 and PAALA-8 contains 6840 and 8136 atoms respectively and therefore several hours of CPU time are required for each geometry optimization step.

RESULTS AND DISCUSSION

X-ray diffraction data

In order to situate adequately this work a recall of some structural data of the compounds under study[9] would be advisable. The X-ray diffraction diagrams obtained from stretched films of PAALA-6 and PAALA-8 without any thermal treatment are displayed in *Figures 2a* and *b* respectively. They indicate the presence of a uniaxial structure made of 13/4 helices spaced out 17 Å and 18 Å respectively and lacking tridimensional order. Density considerations lead to infer that whereas PAALA-8 is presumably organized in layers, the structure of PAALA-6 should be envisaged as a roughly hexagonal packing of helices. A crude representation of what is thought to be the mode of packing of the chains in the structure of each polymer is displayed in *Figure 3*.

The diagram resulting from PAALA-8 annealed at 108°C for a few hours is shown in *Figure 2c* indicating that the polymer has crystallized by effect of the treatment. Such a diffraction pattern may be satisfactorily indexed on the basis of a rectangular lattice with intermolecular distances 18.0 Å and 12.3 Å[9]. The crystal structure is interpreted as an arrangement of sheets made of 13/4 helices with side chains occupying the intersheet space. It should be noted that neither the distance between successive sheets (18.0 Å) nor the interchain distance within the sheets (12.3 Å) are consistent with a fully extended conformation for the side chain. The diameter of the 13/4 helix has been reported to be around 11 Å which added to the 10 Å length predicted for an octyl chain crystallized in the all-*trans* conformation would result in an interchain distance about 21 Å. Therefore side chains must be folded, especially those filling the interchain space in the layers. Furthermore, the side chain is expected to adopt a different conformation for each residue contained in the repeat of the helix since the packing environment fluctuates according to the 13/4 symmetry of the structure.

Hexagonal crystal structure of PAALA-6

The molecular conformation of PAALA-6 packed in the hexagonal form was analysed according to the procedure described in the Methods section. The backbone torsional angles obtained after stages **A** and **B** are listed in *Table 1* where the values used as starting point in the geometry optimization process are also included. Backbone torsional angles reported for other poly(β-L-aspartate)s with a hexagonal structure of 13/4 helices[3,6] are also listed for comparison. Note that conformational parameters obtained for PAALA-6 by energy calculations are very similar to those obtained for the referenced PAALAs by LALS refinement. The fact that the 13/4 helices of PAALA-6 appear to be equally stable suggests that this polymer cannot crystallize in the hexagonal form because the hexyl chains are unable to pack efficiently in a tridimensional structure.

The lattice parameters obtained after stages **A** and **B** are listed in *Table 2*, where parameters inferred from X-ray diffraction data for a hypothetical crystallized structure of PAALA-6 are also included. As can be seen the computed cell parameters differ from the experimentally based values, the largest deviations being 1.5 Å and 4.5°. Such differences indicate that an ordered hexagonal arrangement has not been achieved and that intermolecular distances swing between 17.1 and 17.6 Å. Furthermore, an inspection of the side chain torsional angles reveals deviations from the expected all-*trans* conformation as large at 62°.

Figure 4 shows a projection along the *c*-axis of the unit cell of PAALA-6 obtained from molecular mechanics calculations. Although the 13/4-helical conformation is retained, the helices become tilted with respect to the *c*-axis of the structure. Such arrangement has no precedent among poly(β-L-aspartate)s crystallized in the hexagonal form[2,5,6] and entails a shortening of the *c* axis by about 2.6 Å. It can be inferred therefore that the hexyl chain is too large for stabilizing the hexagonal form.

Table 1 Backbone torsional angles[a] for PAALA-6, PAALA-8 and for other poly(β-L-aspartate)s in the 13/4 helical conformation

Polymer	ϕ	ξ	ψ	ω
PAALA-6 (starting)[b]	145.2	−62.0	130.8	180.0
PAALA-6 (**A**)[c]	145.0	−61.6	130.3	−179.6
PAALA-8 (**B**)[d]	145.0	−61.6	130.3	−179.6
PAALA-8 (starting)[b]	145.2	−62.0	130.8	180.0
PAALA-8 (**A**)[c]	147.8	−58.7	124.4	−179.3
PAALA-8 (**B**)[d]	149.1	−57.7	123.7	179.9
PAIBLA[e]	145.2	−62.0	130.8	180.0
PAALA-4[f]	146.2	−59.8	128.8	180.0
PAMELA[g]	145.4	−59.6	130.0	180.0

[a] The torsional angles (in degrees) correspond to those indicated in *Figure 1*

[b] Torsional angles used in the constrained energy minimizations

[c] Torsional angles obtained from a geometry optimization with the constrained lattice parameters

[d] Torsional angles obtained from geometry optimization without any constraint

[e] Poly(α-isobutyl-β-L-aspartate), ref. 2

[f] Poly(α-*n*-butyl-β-L-aspartate), ref. 6

[g] Poly[α-(2-methoxyethyl)-β-L-aspartate], ref. 6

Table 2 Crystal data for PAALA-6 and PAALA-8 obtained from molecular mechanics calculations

	a[a]	**b**[a]	**c**[a]	α[b]	β[b]	γ[b]
PAALA-6[c]						
Experimental	17.0	29.4	19.0	90.0	90.0	90.0
	17.0	*17.0*	*19.0*	*90.0*	*90.0*	*120.0*
Stage **A**	17.2	31.0	16.7	89.7	85.7	87.4
	17.2	*17.9*	*16.7*	*89.7*	*85.7*	*119.2*
Stage **B**	17.1	30.9	16.7	89.6	85.7	87.4
	17.1	*17.9*	*16.7*	*89.6*	*85.7*	*119.2*
PAALA-8						
Experimental	36.0	24.6	19.9	90.0	90.0	90.0
Stage **A**	36.0	24.6	19.9	90.0	90.0	90.0
Stage **B**	36.0	25.1	19.9	90.6	90.1	90.0

[a] In Å

[b] In degrees

[c] Parameters for the rectangular and pseudohexagonal lattices are in roman and italic respectively

Crystal structure of PAALA-8

The two models considered for the crystal structure of PAALA-8 were refined using the procedure described in the Methods section. Energy calculations revealed that the model with space group $P2_1$ is 3.1 kcal mol^{-1} residue^{-1} less favoured than the model with space group $P2_12_12$. Relative energies calculated for the two packing models are given in *Table 3*. Note than nonbonding interactions are more favoured in the $P2_1$ model than in the $P2_12_12$ one, whereas the bonding contributions are in the reverse order. However, the conformational parameters resulting for the $P2_1$ model (data not shown) reveal that the molecules tend to adopt a distorted conformation abandoning the initial hydrogen bonding scheme. The backbone torsional angles obtained for the 13/4 helix of PAALA-8 in the $P2_12_12$ cell are shown in *Table 1*. Notice that the helix remained stable when the constraints were removed and the values of the torsional angles are similar to those obtained for poly(β-L-aspartate)s packed in the hexagonal form. The variation with respect to the starting values were found to be less than 6° and such deviations are basically due to the folding of the side chains to fit within the lattice.

Lattice parameters derived from geometry optimizations for PAALA-8 are listed in *Table 2*. Due to the symmetry assumed for the lattice, the *a* and *b* cell parameters turn to be the double of the intermolecular distances along the respective directions determined by X-ray diffraction. Comparison with experimental data reveals an excellent agreement since the largest deviations are 0.5 Å and 0.6° for distances and angles respectively. Such differences may be justified considering that many of the force-field parameters used in our calculations were directly transferred from AMBER force-field libraries[11,12], a program which was originally designed for studying proteins and nucleic acids and that we have recently applied with success to the analysis of poly(β-L-aspartate)s[2–5]. Several studies carried out during the last

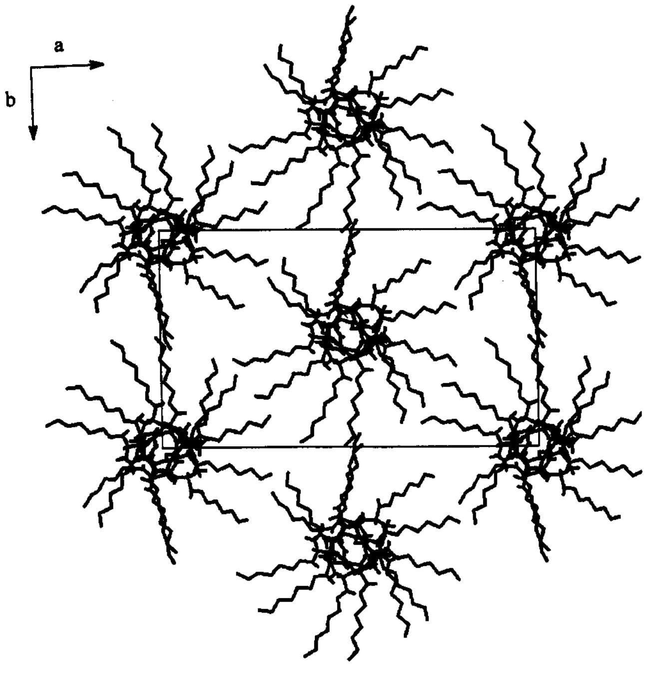

Figure 4 Projection along the *c* axis of the hexagonal unit cell of PAALA-6 obtained from molecular mechanics calculations. Note that the axis of the helices are twisted with respect to the *c* axis

Table 3 Energy contributions[a] (in kcal mol^{-1} residue^{-1}) computed for the different models of the PAALA-8

Space group	E_{bonded}	$E_{\text{non-bonded}}$	E_{TOT}	ΔE^b
$P2_12_12$	6.7	−52.6	−46.1	0.0
$P2_1$	12.2	−55.0	−42.8	3.3

[a] The different energy contributions: E_{bonded} = bonding energy, $E_{\text{non-bonded}}$ = non-bonding energy (electrostatic + van der Waals), E_{TOT} = total energy

[b] Relative energy

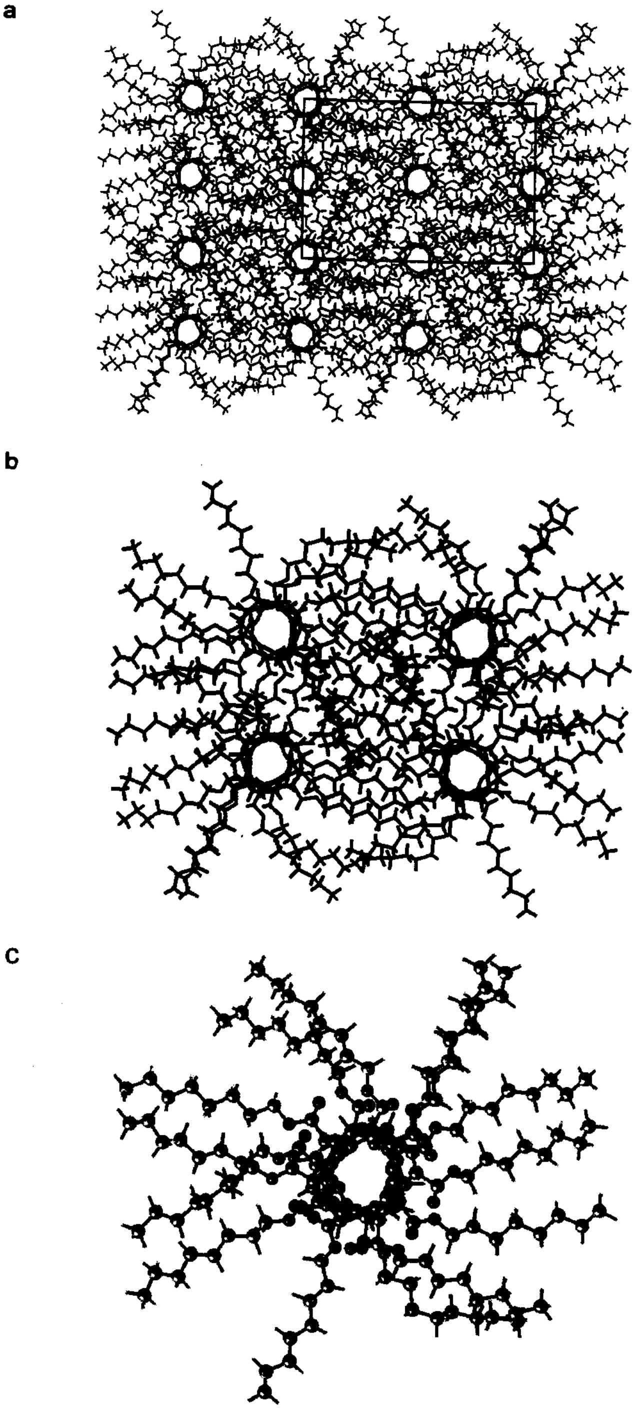

Figure 5 Projection along the *c* axis of the $P2_12_12$ unit cell of PAALA-8 obtained from molecular mechanics calculations: (a) unit cell; (b) four chains; (c) an isolated chain

few years have shown that an explicit evaluation of all the force-field parameters is required in order to obtain an accurate estimation of the structural parameters[13–17].

The final model obtained from energy calculations for the orthorhombic lattice of PAALA-8 is displayed in *Figure 5*. It can be seen that the side chains have to fold in order to provide an efficient mode of packing. The folding is maximum at those residues located within the plane of the sheet and minimum in those protruding from the sheet along a direction near to the normal to it. In *Table 4* the side chain conformational angles for the 13 residues contained in the helix repeat of PAALA-8 are listed, and the molecular geometry of one chain is schematically depicted in *Figure 5c*. Caution must be taken if a quantitative evaluation is to be made since molecular mechanics gives a static picture of just one of the many possible local minima. It is largely expected however that the procedure used in this work permits us to overcome much of the less relevant local minima.

A close inspection of the results listed in *Table 4* reveals that residues of PAALA-8 can be grouped into two classes according to the type of arrangement assumed by their respective side chains. In one class all the torsional angles of the octyl group are near to the *trans* conformation so that the packing efficiency is attained without implying a large distortion of the zig-zag trajectory. Thus, deviations of the conformational angles χ_i, with i ranging from 1 to 10 (see *Figure 1*), from the standard *trans* values ($\chi_i = 180°$) appear to be less than 40°. The second type of arrangement corresponds to those residues in which one dihedral angle of the side group adopts a *gauche* conformation giving rise to an abrupt change in the trajectory of the chain. These residues are written in bold in *Table 4*. Note that in all cases the *gauche* conformation is taken by a bond relatively close to the ester group. A joint inspection of *Figure 4* and *Table 4* further reveals that the two types of arrangements described above correspond to residues located near the *a*- and *b*-directions respectively.

An approximately quantitative picture of the conformational behaviour of the side chains in the orthorhombic lattice of PAALA-8 is given by the bar graph depicted in *Figure 6a*. In such a diagram the number of residues with the methyl carbon atom of the side chain (C15 in *Figure 1*) at a distance r from the axis of the helix are represented for intervals of 0.5 Å. The distribution profile may be divided into three regions which are the position residue around the helix. The first region comprises r values between 12.25 Å and 12.75 Å and embraces those residues located in the plane of the sheet. Since the side chains of these residues are severely folded, the distances from the methyl to the axis of the helix are considerably shortened. The second region with r ranging from 12.75 Å to 13.75 Å includes residues positioned at half way between the intersheet and sheet directions. Finally, the region with r fluctuating between 13.75 Å and 14.75 Å contains the residues located out of the sheet plane. Although in the latter region the alkyl side chains are essentially extended, comparison of r with the value predicted for an ideal *trans* conformation [8 × 1.25 Å (length of the octyl chain) + 5.5 Å (radius of the helix including the carboxylate group) = 15.5 Å] indicates a shortening of about 1.0–1.5 Å. Such contraction can be explained in terms of side chain torsional angles if the above-mentioned deviations from the ideal 180° value are taken into account (see *Table 4*).

Packing of the side chains in PAALA-8

X-ray diffraction data for PAALA-8 indicate that the layers in which helices are arranged are spaced out 18.0 Å. Our calculations indicate however that the distance between the axis of the helix and the end of the side chains ranges from 12.25 Å to 14.75 Å depending on the position of the residue around the contour of the 13/4-helix. This implies that side chains of neighbouring molecules must interpenetrate in order to attain an efficient mode of packing.

An overall picture of the packing of the side chains of PAALA-8 is given by the representation of the number of residues as a function of the distance d between the methyl carbon of the side chain (C15 in *Figure 1*) and the closest backbone atom of the neighbouring chains. The bar graph is shown in *Figure 6b* which indicates that such distances range from 3.0 Å to 7.0 Å and where each block represents a 1.0 interval in distance d. Whereas more than half of the side chains have d values between 3.0 Å and 5.0 Å, the remaining side chains spread over a wider interval ranging from 5.0 Å to 7.0 Å. The former group

Table 4 Conformational angles[a] for 13 residues comprised in four turns of the helical structure of PAALA-8

No.[b]	χ_1	χ_2	χ_3	χ_4	χ_5	χ_6	χ_7	χ_8	χ_9	χ_{10}
1	−172.3	175.6	152.3	−172.9	168.9	−172.1	171.3	−171.7	172.3	174.7
2	170.6	172.9	120.3	169.0	158.3	−150.0	179.0	174.0	176.9	173.4
3	149.6	−166.2	157.1	161.4	−170.6	66.5	−165.2	−176.9	−167.6	−173.9
4	168.5	169.3	157.4	155.8	77.5	−161.4	−165.9	−166.2	−162.4	174.0
5	−175.2	175.1	157.4	178.4	175.7	140.2	178.5	−174.0	−177.5	−163.9
6	171.6	175.2	163.3	−166.9	177.0	−170.0	−173.4	−166.3	−172.9	177.4
7	−154.4	−176.8	167.6	−162.0	76.9	173.3	179.0	180.0	−175.9	−169.0
8	−173.8	−175.5	148.2	179.9	−78.2	168.8	179.9	179.1	180.0	−176.9
9	178.6	179.3	176.4	−170.7	−165.1	−172.1	168.4	−167.8	−176.3	−169.5
10	−173.4	170.6	163.3	−160.9	170.6	−161.8	165.0	−157.1	175.5	−175.4
11	152.6	−160.0	117.1	170.2	−155.0	174.6	−172.1	−174.8	−179.3	172.4
12	−178.5	171.4	161.1	−61.2	160.8	−173.3	−178.1	178.8	−175.8	168.1
13	−175.1	−177.2	148.4	158.2	−172.3	177.8	−174.4	−179.5	−170.7	−167.5

[a] In degrees

[b] Numbers in bold correspond to those residues in which one dihedral angle of the side group adopts a *gauche* conformation

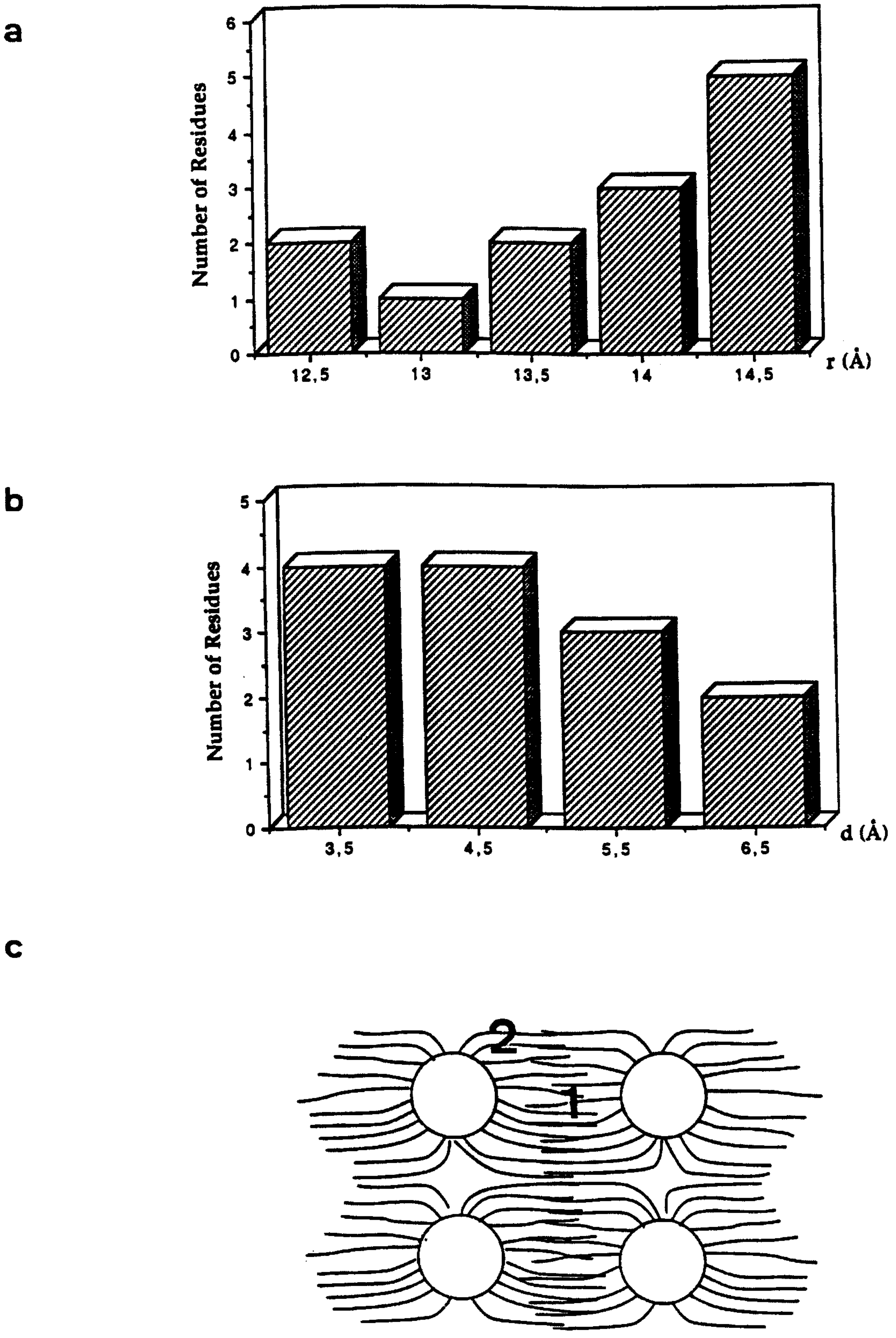

Figure 6 Bar graph representations of the statistical results for the arrangement of the side chains of PAALA-8 in the crystallized state. (a) Frequency of residues with a distance *r* between the end of the side chain and the helical axis. (b) Frequency of residues with a distance *d* between the end of the side chain and the closest backbone atom of the neighbouring chains. (c) Schematic representation of the side chain conformations classified according to statistical results

comprises those residues with attached side chain penetrating up to the proximity of the ester group of the neighbouring molecule; such residues are those located near to the intersheet direction and are indicated as **1** in the scheme of *Figure 6c*. Conversely side chains on residues placed near to the sheet plane hardly interact with neighbouring chains. They are labelled as **2** in *Figure 6c*. Between the two extreme situations of minimum and maximum penetration, a number of intermediate states exist as can be noted from the broad set of values obtained for *d*.

The results attained by means of this approach are in full agreement with those attained by measuring the distance between the side chain end and the helix axis. The concordance observed for the two methods gives support to the complex scheme trajectories resulting from energy calculations and corroborates that penetration of the side chains into the molecular space of the neighbouring molecules depends largely on the position of the residue within the 13/4 helix.

Layered crystal structure of PAALA-6

The model proposed for PAALA-8 has been used to investigate the stability of an hypothetical layered structure of PAALA-6. The structure has been generated by removing two methylene units in the all-*trans* part of the PAALA-8 side chains and reducing the cell dimensions accordingly. Geometry optimizations were performed according to the two stage process described in the Methods section. In this case, the resulting structure remains stable with the helices parallel to the *c*-axis of the structure. Lattice parameters resulting for the $P2_12_12$ unit cell derived from geometry optimizations were $a = 3.14$ Å, $b = 25.6$ Å and $c = 19.5$ Å. Comparison between the hexagonal and the layered structures computed for PAALA-6 indicates that the former is favoured by about 6 kcal mol^{-1} residue^{-1}. This is obviously the artefactual result due to the distorted structure obtained for the hexagonal form.

CONCLUDING REMARKS

The results presented in this work give a comprehensive picture of the structure and conformational behaviour of PAALA-6 and PAALA-8. Since the complexity of the systems precludes the use of LALS refinement for the determination of the conformational parameters, energy calculations have been used to obtain an approximate description of the structure of these poly(β-L-aspartate)s. Several conclusions concerning the packing of both helical main chains and side chains may be drawn from the results presented in the foregoing paragraphs.

(a) The size of the hexyl side chain is revealed to be inadequate to stabilize the hexagonal crystal structure characteristic of poly(β-L-aspartate)s bearing short alkyl side chains. However, our results reveal that the size of this group is compatible with a layered structure similar to that observed in polymers with large alkyl side groups. This result does not allow us to understand the fact that PAALA-6 cannot be crystallized.

(b) The $P2_12_12$ space group appears to be the most favoured arrangement for the orthorhombic structure of PAALA-8. In such a structure the unit cell contains two pairs of antiparallel right-handed 13/4-helices with side chains more or less folded depending on their position along the helix. Two different patterns of bending have been observed for side chains. In one of them, the turn of the chain occurs smoothly with all the C–C dihedral angles deviating slightly from the *trans* conformation. In the other, a specific dihedral angle adopts a *gauche* conformation whereas the other remains practically unaltered; as a consequence, the trajectory of the polymethylene chain changes abruptly at that point.

(c) Side chains of PAALA-8 interpenetrate along the intersheet direction in an extent that is depending on the position of the residue along the helix. Side chains coming out from both sides of the sheets extend up to the proximity of the ester groups placed on neighbouring sheets. On the other hand, interactions between side chains within the same sheet are almost negligible.

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