Electron Microscopy and Structural Modeling of the 3-Fold Helical Form of Poly(glycyl-β-alanine)

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ABSTRACT: Triangular lamellae of poly(glycyl-β-alanine) in form II with chains folded back and forth were obtained by crystallization from dilute solutions. A structural modeling analysis of this crystalline form was carried out with the LALS program. Electron diffraction and crystal morphology data were used to establish the symmetry of the structure. A model consisting of a statistical arrangement of up and down helices in a 3-fold helical conformation similar to polyglycine II is proposed. The unit cell is pseudohexagonal (a = 4.79 Å, c = 22.2 Å) and contains two chains arranged in opposite orientation, each one having an occupancy of one-half.

Introduction

The crystalline model known as polyglycine II was put forward by Crick and Rich in 1955.[1] This consists of a hexagonal array of 3-fold helices intermolecularly linked by a three-dimensional net of hydrogen bonds. Although such a structure is seldom observed for polypeptides, its biological significance is highly relevant, as a similar type of helix is found for protein chains in collagen.[2] Despite this, a precise description of the polyglycine II crystal model has not been given so far and ambiguities concerning both the helix handedness and the up-to-down arrangement of chains still persist. Failure to obtained well-oriented samples with this structure appears to be the major drawback preventing its rigorous characterization.

We have recently discovered that certain regular copolyamides containing glycine are also able to adopt the polyglycine II crystal model.[3-5] These findings showed a departure from the common crystalline habit exhibited by synthetic polyamides. They demonstrated also that a fully polypeptide configuration is not indispensable for the occurrence of such structures. These issues add a renewed interest to the investigation of this subject.

Poly(glycyl-β-alanine) is a regular alternating copolyamide with a chemical constitution intermediate between polyglycine and nylon 3

$$(NHCH_2CONHCH_2CH_2CO)_n$$

Two crystalline forms were found for this polymer.[3] Whereas form I has the layered organization typical of nylons, form II is a hexagonal lattice similar to polyglycine II. A unit cell (a = 4.79 Å, c = 22.2 Å) containing one chain in 7*3/2 helical conformation was proposed for this second form. As no evidence for up-to-down arrangement of chains was obtained, a simple model with helices oriented in parallel was then assumed.

We have now succeeded in obtaining single crystals of poly(glycyl-β-alanine) in form II. Electron microscopy of these crystals has provided further valuable information which affords a better understanding of the crystal structure present in this form. On the basis of such new data together with those previously provided by X-ray, a modeling analysis has been carried out with the LALS program.[6] Several possible models were examined and compared. Our selection included alternative arrangements differing either in the molecular conformation or in the side-by-side packing adopted by the chains in the crystal.

Experimental Section

A sample of poly(glycyl-β-alanine) of $M_n \approx 6300$ corresponding to a $\bar{X}_n$ of about 50 was used for this study. The synthesis and chemical characterization of this polymer were described in detail elsewhere.[3]

Crystallization was carried out from dilute solutions of the polymer in mixtures of water–formic acid. Crystals were collected by centrifugation and washed repeatedly with volatile alcohols. Samples for electron microscopy were prepared in the usual way and shadowed with platinum–carbon at an angle of 14°. Selected area electron diffraction diagrams were recorded at 100 kV on Kodak Tri-X films. The patterns were internally callibrated with gold (d_{111} = 2.35 Å). X-ray diffraction diagrams were registered from dried sediments of crystals at room temperature. A modified Statton camera (W. R. Warhus, Willmington, DE) with graphite-filtered copper radiation was used for this work.

Results

Electron Microscopy. Rather complex crystals displaying a predominant triangular shape were instantaneously formed when 20 volumes of water were poured into an 1% solution of poly(glycyl-β-alanine) in formic acid at room temperature (Figure 1). These morphologies were too thick to be examined by electron microscopy. If addition of water was carried out at 96 °C instead, precipitation of the polymer proceeded smoothly after a few minutes. Well-developed triangular lamellae with a thickness of about 40 Å (when measured in micrographs of shadowed samples) were produced under such conditions (Figure 2A). The X-ray diagram from a sediment of these lamellae contains all the reflections characteristic of form II. Two rings with Bragg spacings at 44 and 22 Å were in addition observed in the low-angle X-ray diagram recorded from that sample. They are interpreted as the first- and second-order reflections arising from the stacking of lamellae 44 Å thick.

A hexagonal pattern with a basic spacing at 4.15 Å and exhibiting $6/mmm$ symmetry was recorded by selected area electron diffraction from one isolated lamella (Figure 2B). Up to four orders of the 4.15 Å reflection can be seen in the original picture, which indicates that the crystal structure is well preserved to near 1-Å resolution. This pattern clearly corresponds to the projection down the c-axis of the crystal lattice of form II. Consequently, chains in the crystal must stand with their axes not far from normal to the surface. Provided that a 7*3/2 conformation is followed by the polymer, its computed average length turns out to be nearly 10-fold the value of the lamellar thickness. It can be reasonably inferred from such a ratio that the polymer chain must fold back and forth in order to be accommodated within the crystals.

Two extreme cases can be conceived for a crystal made up-and-down chains: (i) a true crystal consisting of a regular up-and-down arrangement of chains and (ii) a pseudocrystal where chain orientations are at random. In the first case a unit cell containing at least two chains in

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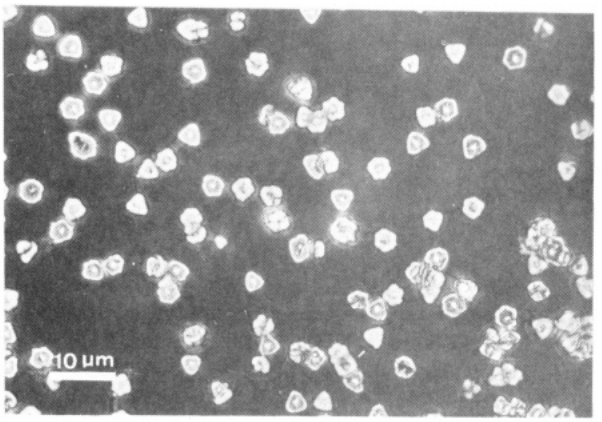

Figure 1. Optical micrograph of a suspension of crystals of poly(glycyl-β-alanine) in form II.

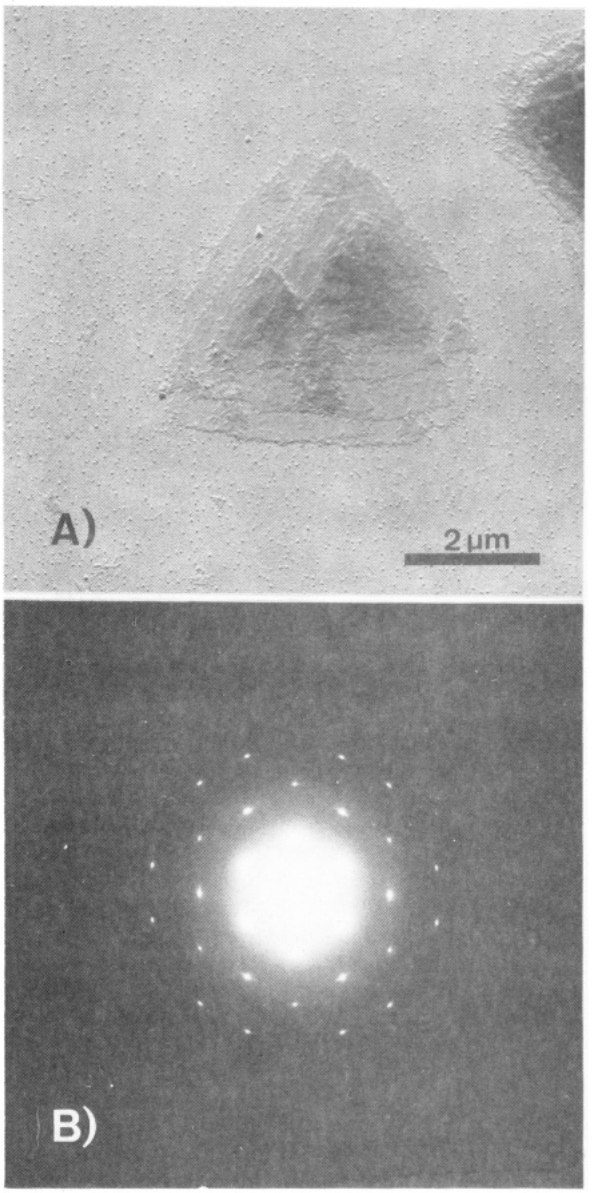

Figure 2. Triangular lamellae of poly(glycyl-β-alanine) in form II (A) and its corresponding electron diffraction diagram (B).

opposite direction should be needed to describe the structure properly. In the second case a pseudohexagonal cell with $a = 4.79$ Å would be valid if two chains in opposite orientation are considered to be superimposed within such a cell. The occupancy for each chain should be one-half in order to satisfy density packing considerations.

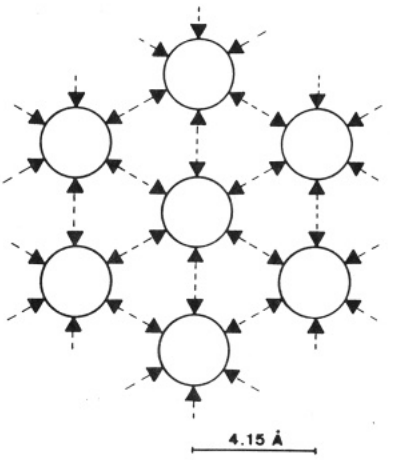

Figure 3. General scheme of the projection of the polyglycine II crystalline structure down the helix axis. Only locations for CO and NH groups are shown. Hydrogen bonds are represented by dashed lines.

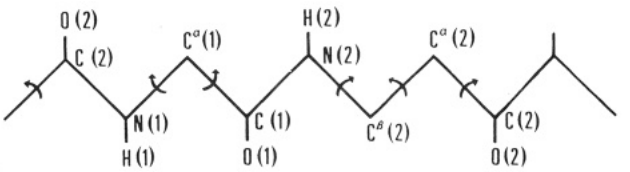

Figure 4. Representation of the glycyl-β-alanyl backbone showing atom numbering and definition of torsional angles as used in Tables I and III.

Structural Modeling. Polymers with polyglycine II structure cannot be stretched into fibers and this is the case also for poly(glycyl-β-alanine). Nevertheless, diffraction data recorded from unoriented samples and films grown epitaxially on hydroquinone revealed that a 3-fold helical conformation is adopted also by this polymer as was reported in an earlier paper.[3] A chain made of glycyl-β-alanyl units related by 3_2 symmetry and with a translation height of 7.4 Å was derived by comparison with polyglycine II. The hexagonal lattice with $a = 4.79$ Å results, therefore, when every helix is bridged by hydrogen bonds to its six neighbors, as is shown in Figure 3.

According to these finds the glycyl-β-alanyl residue was taken as the basic unit to generate a helix with a 3-fold screw axis. Standard bond distances and bond angles for polypeptides and polyamides were adopted to build the repeating unit and kept constant throughout the whole modeling process. Only the five torsional angles (which are indicated in Figure 4) and the positional parameters fixing the chain in the unit cell were affected by the refinement.

The symmetry elements of the basal plane of the structure become defined by the electron diffraction diagrams arising from single crystals (Figure 2b). Three planar point groups ($p6m$, $p3m1$, $p31m$) are consistent with the $6/mmm$ symmetry exhibited by the $hk0$ hexagonal pattern. A unit cell containing six glycyl-β-alanyl residues is required by both $p31m$ and $p3m1$ groups, which would result in a density packing twice as high as the expected value for a poly(glycyl-β-alanine) structure having a c repeat of 22.2 Å and an interchain distance of 4.79 Å. Such incongruency vanishes if the statistical arrangement of up-and-down chains considered above is assumed to occur in the crystal. By this approach, a unit cell able to fulfill the symmetry requirements of both groups can be defined. As stated above, such a cell would consist of two superimposed 3/2 helices in opposite orientation. On the other hand, the $p6m$ choice is highly unlike because 12 residues

Table I
Distances and Angles for Hydrogen Bonds between Parallel and Antiparallel Chains of Poly(glycyl-β-alanine) II

atom	chain	residue	atom	chain	residue	N-O dist, Å	OHN, deg	NOC, deg
				Parallel				
O(1)	up(0,0,0)	1	N(2)	up(1,1,0)	1	2.73	170	171
O(2)	up(0,0,0)	-1	N(1)	up(-1,0,0)	1	2.88	156	165
				Antiparallel				
O(1)	down(0,0,0)	1	N(1)	up(1,1,0)	3	2.86	144	161
O(2)	down(0,0,0)	-1	N(2)	up(0,-1,0)	3	2.73	142	171

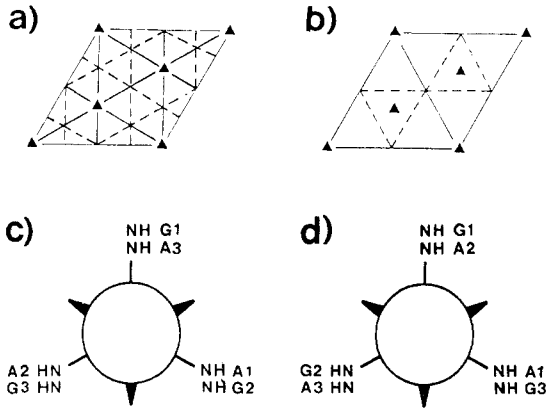

Figure 5. Schemes of plane groups $p3m1$ (a) and $p31m$ (b) indicating the location for 3-fold screw axes by triangles and 2-fold axes by solid lines. Projections of the conformational models B (c) and C (d) (see the text) for the right-handed helix of poly(glycyl-β-alanine) II. Application of arrangement (d) on the $p31m$ lattice (b) results in a structure having only three equivalent hydrogen-bond directions coincident with the three binary axes.

in the unit cell are needed to satisfy the symmetry of this group.

$p3m1$ and $p31m$ differ one from the other in the location of the three 2-fold axes normal to the chain axis which are present in both lattices (Figure 5). Such difference in symmetry implies that a distinct helical arrangement must be followed by the chain in each case if all hydrogen bonds are to be formed. A lattice with three equivalent hydrogen bond directions coincident with the three bindary axes may be generated with $p31m$ symmetry if a convenient arrangement of CO and NH groups is set along the helix. On the other hand, a lattice having six equivalent hydrogen bond directions is the only one compatible with the $p31m1$ symmetry.

If, as is well-known, crystallization kinetics determines the crystal face growth, the differences between $p31m$ and $p3m1$ pointed out above should be reflected in the crystalline morphology displayed by the polymer. Whereas a hexagonal habit may be expected from either $p3m1$ or $p31m$, only the latter is able to account for the development of triangular crystals such as those observed in this work. Therefore, our subsequent analysis will be confined to this case.

Three alternative arrangements are feasible for a 3-fold poly(glycyl-β-alanine) chain having CO and NH groups aligned in specific rows along the helix. They differ in the rotation angles which are set for the glycyl (w_G) and β-alanyl (w_A) residues when considered as independent units. These angles are defined in projection by taking the nitrogen atoom of each residue as reference.

Model A: $w_G = 0$; $w_A = 120$. The modeling analysis reveals severe stereochemical hindrances for this arrangement. Therefore this model may be definitely rejected.

Model B: $w_G = 120$; $w_A = 0$. Although this model appears plausible on stereochemical grounds, it turns out to be inadequate when observed and calculated X-ray data are compared. In particular, an inadmissible structure factor is computed for the 4.15-Å reflection, which does not compare at all with the observed value. In fact, this reflection appears prominent in every diffraction diagram recorded from poly(glycyl-β-alanine) II as well as in all those arising from polymers with the polyglycine II structure.

Table II
Observed (F_o) and Calculated (F_c) Structure Factors for the Model of Poly(glycyl-β-alanine)[a] II

ring	d(obsd), Å	F_o	hkl	d(calcd), Å	m[b]	F_c
1	7.40	30	003	7.40	1	28
2	4.10	202	100	4.15	3	163
			101	4.08	3	
			001		3	
3	3.89	142	102	3.89	3	193
			012		3	
4	3.64	99	006	3.70	1	76
			103	3.62	3	
			013		3	
5	3.30	141	104	3.32	3	117
			014		3	
6	3.03	72	105	3.03	3	86
			015		3	

[a] No temperature factor ($B = 0$) was used. Only the scale factor was refined. The X-ray discrepancy indexes $R = (\sum|f_o - F_c|)/(\sum|F_o|)$ and $R^4 = \{(\sum|F_o - F_c|^2)/(\sum|F_o|^2)\}^{1/2}$ are 22.6% and 24.0%, respectively. F_c is calculated as $F_c = (\sum m_{hkl}F_{chkl}^2)^{1/2}$, where the summation extends to all reflexions contained in the ring. [b] Multiplicity.

Model C: $w_G = w_a = 120$. This is actually the model we proposed in our previous report.[3] As model B, it appears stereochemically suitable and shows no significant contacts. All hydrogen bonds are allowed to be formed with length and angle values within the standard ranges (Table I). The model has not be refined against X-ray data because of the poor quality of available diagrams and only the scaling factor was processed. Nevertheless an acceptable concordance between observed and calculated data is attained, with no large discrepancy for any particular reflection (Table II). In conclusion, this arrangement appears as the most favored for the poly(glycyl-β-alanine) chain in form II. Molecular drawings of the parallel and antiparallel arrays of chains built according to this model as well as the construction which results when two chains in opposite direction are superimposed in every position are given in Figures 6 and 7. Torsion angles and coordinates for the atoms of the chain are listed in Table III.

Discussion

Crystallization of poly(glycyl-β-alanine) from solution invariably takes place in form II, and no signs of form I has been detected in these experiments. It becomes obvious that the polymer strongly prefers to take up a helical conformation rather than to be organized in extended chain layers. It is in such a way as a molecule can interact strongly with its six surrounding neighbors. This trend is in fact shared by all those polymers which are able to adopt the polyglycine II structure.

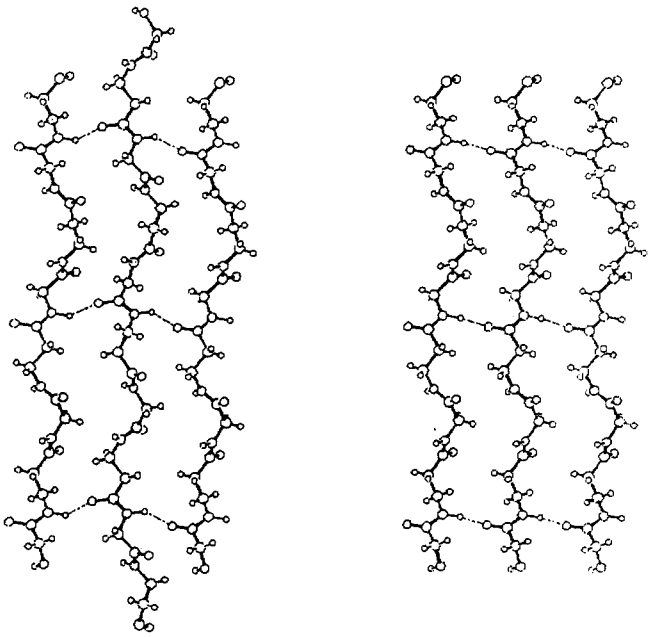

Figure 6. Side view of three neighboring molecules of poly(glycyl-β-alanine) II drawn with the ORTEP program.[11] All hydrogens atoms are shown. Hydrogen bonds linking these molecules are represented by dashed lines. The relative chain orientation is up-down-up in (a) and up-up-up in (b).

Table III
Cylindrical Coordinates and Torsion Angles for the Poly(glycyl-β-alanine) II Model (Right-Handed Helices, Helix Axis at $x = 0$, $y = 0$)[a]

	molecule up			molecule down		
	r, Å	ϕ, deg	Z, Å	γ, Å	ϕ, deg	Z, Å
			Cylindrical Coordinates			
O(2)	2.18	163.3	−13.26	2.18	−103.3	6.02
C(2)	1.01	177.6	−13.42	1.01	−117.6	6.18
N(1)	0.13	95.7	−12.57	0.13	−27.9	5.34
H(1)	1.02	−21.9	−12.72	1.02	81.9	5.49
$C_\alpha(1)$	0.96	95.7	−11.36	0.96	−35.7	4.12
O(1)	2.12	21.5	−10.12	2.12	38.5	2.89
C(1)	0.90	25.1	−10.27	0.90	34.9	3.04
N(2)	0.62	−64.2	−9.52	0.62	124.2	2.28
H(2)	1.18	−129.8	−9.65	1.18	−170.2	2.42
$C_\beta(2)$	1.58	−49.1	−8.44	1.58	109.1	1.20
$C_\alpha(2)$	1.14	−12.0	−7.24	1.14	72.0	0.0
			Torsional Angles			
$C(1)-C_\alpha(1)-N(1)-C(2)$					151.4	
$N(2)-C(1)-C_\alpha(1)-N(1)$					−87.1	
$C_\alpha(2)-C_\beta(2)-N(2)-C(1)$					76.4	
$C_{i+1}(2)-C_\alpha(2)-C_\beta(2)-N(2)$					119.7	
$N_{i+1}(1)-C(2)-C_\alpha(2)-C_\beta(2)$					−147.9	

[a] Coordinates for the left-handed model are readily obtained by inverting the sign of Z.

In spite of its rarity among polymers, a triangular crystal habit has been already described for several related polyamides.[4,7] The thickness of 44 Å measured for these crystals correlates well with those values found for other polymers crystallizing in the same structure.[4,5,7] Similar values were also reported for single crystals of polypeptides in β form.[8] Electron diffraction diagrams are readily recorded from these triangles, whose exceptional stability under the electron flux is very likely due to the existence of the hydrogen bond network which tightens the whole crystal.

The ability of polyglycine II chains to generate a lattice either in parallel or in antiparallel arrangement was noted as early as this structure was described for the first time.[1]

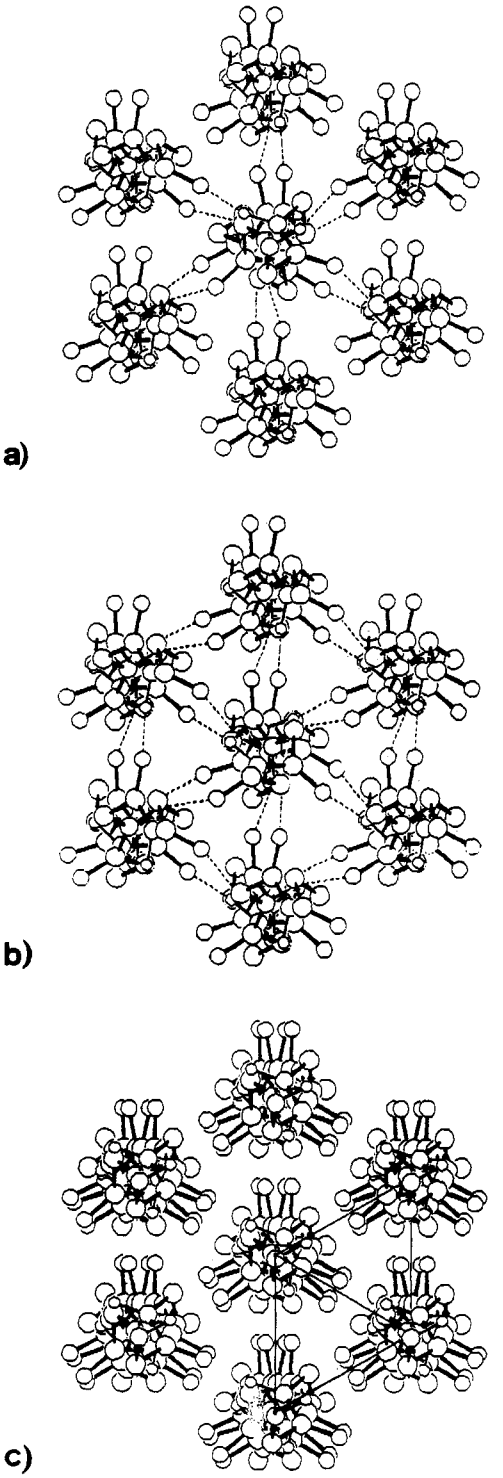

Figure 7. Down the c-axis view of a central molecule and its six surrounding neighbors. The orientation of the central molecule with respect to the up-pointing neighbors is down in (a) and up in (b). Only hydrogen atoms involved in hydrogen bonds are represented. Drawing c corresponds to an ideal structure which has been constructed by placing two chains in opposite direction in every position of the lattice. The resulting bidimensional packing has $p31m$ symmetry.

Evidence of the occurrence of up-and-down chains came later from electron microscopy observations made on hexagonal lamellae by Padden and Keith[7] and also from infrared spectroscopy.[9] A structural analysis supporting such observations was then carried out by Ramachandran.[10] However, a detailed model including a precise description of the arrangement of chains in the cell has not yet been offered.

It should be kept in mind that a hexagonal array made of up-and-down chains implies both parallel and antiparallel interactions between neighbors. The approach used here for the modeling analysis rests on the assumption that up-and-down chains are localized at random in the crystal. This is in agreement with the symmetry exhibited by the single-crystal electron diffraction pattern. Although adjacent reentry of an individual molecule will generate an alternating antiparallel row of about 10 stems, the existence of such short-range arrangements should not modify substantially the statistical model.

An orthorhombic lattice (with triclinic symmetry) would arise, on the other hand, if a long-range regular arrangement of up-and-down chains were adopted. However, no signs of symmetry other than hexagonal were detected in the electron diffraction patterns.

The triangular crystalline habit displayed by this polymer is a feature which has allowed us to decide between $p31m$ and $p3m1$ packing models. A similar reasoning could be applied to poly(glycyl-β-alanyl-β-alanine), a polymer crystallizing in triangular lamellae highly resemblant to those described here. It is not, however, the case for other polymers with polyglycine II structure. Thus, nylon 2/6, nylon 1-3, and polyglycine itself crystallize as hexagons so that no clear conclusions about chain conformation can be drawn from the crystal habit in these cases.

The results of our modeling analysis confirm the 3-fold helical conformation for the poly(glycyl-β-alanine) chain. The prediction that the glycyl residue is arranged as in polyglycine II and that a gauche conformation is imposed on the methylene pair of the β-alanyl unit is also corroborated. A planar group $p31m$ is proposed for the packing of the 3-fold helices. The three 2-fold axes normal to the helix which are required by the group symmetry are created by superimposing two lattices made of helices pointing to opposite directions (Figure 7c). This is in fact a pseudocrystal having, on the average, the symmetry of space group $P3_121$.

Model building has been carried out assuming right-handed helices, but a left-handed model should be equally valid as no asymmetric carbon exists in the molecule. However, the helix sense must be kept the same within a given crystallite in order to enable the formation of all hydrogen bonds.

Finally we would like to remark that the building procedure mainly relies on stereochemical and symmetry considerations and only a qualitative evaluation of diagrams has been taken into account. No refinement against diffraction data was undertaken. Nevertheless, conclusions drawn from this study suffice to justify the adequacy of the proposed model.

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Registry No. Poly(glycyl-β-alanine) (homopolymer), 83891-62-7; poly(glycyl-β-alanine) (SRU), 115678-01-8.

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Isotactic Polystyrene Phase Diagrams and Physical Gelation

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ABSTRACT: A partial phase diagram was determined for fully isotactic polystyrene of molecular weight 160 000 in nitrobenzene, iPS/NB. Three distinct ways were found in which phase separation occurred; each mode of phase separation was accompanied by a unique gelation process. In region I iPS crystallized from solution, in region II super-cooled iPS solutions underwent a liquid-phase separation followed by iPS crystallization, and in region III the solvent, NB, froze. In both regions I and II gels formed only after phase separation. In region III a gel would form if NB was allowed to thaw. Hence, gels could be formed in three distinct ways. All the modes of gelation resulted in supermolecular structures which were determined by the kineics of phase separation. Subsets of the three mechanism were found operative with other solvents for iPS.

Introduction

Although the gelation of isotactic polystyrene (iPS) solutions has been studied for a long time,[1] little, if any work has been done to determine the phase diagrams of these solutions. This is surprising because a variety of gelation mechanisms have been put forth, all of which are related to different types of phase separation. Hence, knowledge of the phase diagram would help to elucidate the various mechanisms that have been proposed. These mechanisms include fringed micellar crystallization,[2] liquid–liquid phase separation,[3] and even a stable thermodynamic phase for the gel.[4]

The gelation of other semicrystalline polymers has been studied and mechanisms proposed for the observed phenomena. The gelation of poly(4-methyl-1-pentene) by linking of growing spherulites[5] has been described.

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