1540 *Macromolecules* 1989, *22,* 1540–1545

Electron Microscopy Study of Crystalline Polymorphism of Helical Polyamide Poly(α-isobutyl L-aspartate)

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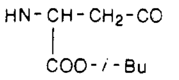
Received July 26, 1988; Revised Manuscript Received October 26, 1988

ABSTRACT: The two helical crystalline forms known to exist for poly(α-isobutyl L-aspartate), hexagonal and tetragonal, were characterized by electron microscopy of crystals grown in dilute solution. The polymer crystallized from chloroform as large hexagonal or semihexagonal thin lamellar crystals, which yielded high-resolution electron diffraction patterns corresponding to the hexagonal form. Small square-shaped crystals as well as stacks of lamellae lying on edge were instead obtained when the polymer crystallized in the tetragonal form. Electron diffraction data obtained from the latter were consistent with the 4/1 helical model proposed earlier for this form. Heat treatment of the hexagonal lamellae at about 200 °C promoted the appearance of the tetragonal form accompanied by thickening of the crystals. A further conversion into a poorly defined hexagonal structure was observed when the crystals were annealed at 270 °C. Possible mechanisms for such changes are discussed in terms of both micromorphology and molecular structure.

Introduction

Poly(α-isobutyl L-aspartate), abbreviated PAIBLA in the text, is a nylon-3 derivative capable of taking up helical conformations similar to the α-helix or polypeptides.[1] It consists of a poly(β-alanine) backbone with a carboxylate group stereoregularly attached to every repeating unit.



$$\underset{\displaystyle \mathrm{COO\text{-}\mathit{i}\text{-}Bu}}{\mathrm{HN\text{-}\underset{|}{CH}\text{-}CH_2\text{-}CO}}$$

We have recently reported that PAIBLA may adopt two crystalline forms in the solid state.[2] The hexagonal form, which actually fits an orthorhombic lattice, consists of 13/4 helices hexagonally packed in a cell of parameters a = 13.5 Å, b = 23.4 Å, and c = 19.9 Å containing two chains in antiparallel arrangement. The structure of this form was determined on the basis of data provided by X-ray diffraction of fibers easily prepared by streching the polymer from a solution in chloroform. The tetragonal form appears when the polymer is precipitated from chloroform by adding ethanol. The molecular model for this latter form was established from powder X-ray diagrams, as no oriented samples with such structure could be obtained. A tetragonal lattice made of helices having four residues per turn with a = 14.0 Å and c = 4.95 Å is consistent with the available data. A scheme showing the mode of packing of the helices in each of these forms is presented in Figure 1. Both forms have identical calculated densities.

The purpose of the present work is to characterize the two crystalline polymorphs of PAIBLA by electron microscopy. Our study has been mainly made on lamellae of the polymer obtained by crystallization from dilute solutions. New data have been obtained that provide strong support for the earlier proposed models and unravel new aspects of the crystalline morphology of PAIBLA. We have also studied the changes observed, in both morphology and crystalline structure, when single crystals of PAIBLA are heated at temperatures in the 100–270 °C range. They demonstrate that a hexagonal–tetragonal transition takes place in the solid state in this polymer.

Experimental Section

PAIBLA was synthesized by polycondensation of active esters using L-aspartic acid as raw material by the method described by Yuki et al.[3] with some slight modifications. It was fractionated by precipitation from chloroform by adding ethanol and extensively characterized by chemical and spectroscopic methods as described elsewhere.[2] Its content in either enantiomeric D units or β-linked units was assessed to be lower than 5%, and no polyimide units were detected. Samples with intrinsic viscosities in the range 0.50–0.30 dL/g (dichloroacetic acid, 25 °C) were used for this work. Average molecular weights between 60 000 and 30 000 are estimated for these samples when the viscosimetric equation derived for poly(benzyl L-glutamate) is applied.[4] It can be easily derived that a hexagonally packed 13/4 helix with the dimensions calculated from the X-ray pattern[2] would have a length of 448 Å for a molecular weight of 50 000.

For X-ray diffraction, films about 100 μm thick were cast at room temperature. X-ray diagrams from such films oriented normally to the beam were recorded at room temperature and under vacuum. A modified Statton camera (W. H. Warhus, Wilmington, DE) mounted on a generator provided with a graphite monochromator and working with copper radiation was used for these experiments. Crystallizations were made from solutions at concentrations around 0.1%. The suspensions containing the grown crystals were evaporated between two glass slides and shadowed with platinum–carbon at an angle of 14°. The adhered crystals were stripped off and examined in a Philips EM-301 instrument operating at 100 kV. Electron diffraction diagrams were recorded from selected areas and internally calibrated with gold (d_{111} = 2.35 Å).

Results

Cast Films. Films made of polymer in hexagonal form were obtained by casting in chloroform; their X-ray diagrams contained the characteristic reflections associated with such form, in particular the most intense spacing, 11.7 Å (Figure 2A). Films cast in 2-chloroethanol yielded highly crystalline patterns corresponding to the tetragonal form, with an inner intense reflection at 14.0 Å in this case (Figure 2B). Films produced with trifluorethanol as solvent gave diagrams containing reflections at both 11.7 and 14.0 Å, which could be satisfactorily indexed if a mixture of hexagonal and tetragonal forms was assumed to be present in such films (Figure 2C).

Hexagonal Form. Thin hexagonal platelets were grown from a 0.1% solution of PAIBLA in a mixture of chloroform–ethanol (3:1) stored for several weeks at 4 °C. The crystals were fairly stable while in suspension but turned out to be extremely sensitive to environmental modifications. They were destroyed not only when the solvent was removed by filtration or centrifugation but also when replaced by other solvents such as pure ethanol or ethyl ether. Specimens for electron microscopy had to be prepared by slow evaporation of the suspension between two glass slides. Crystals then adhered to the glass surface, and their structure was reasonably preserved. Other morphologies were originated during this process, and some additional material without any definite morphology was

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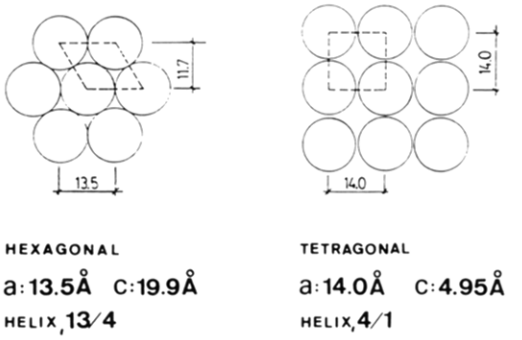

Figure 1. Chain packing of PAIBLA in the hexagonal and tetragonal forms.

also present in the preparations. As a consequence, different crystalline morphologies could be distinguished when examined under the electron microscope. The three most representative types are shown in Figure 3: (A) *overgrown lamellae*, displaying striations parallel to the edges, (B) *sectorized hexagons*, subdivided into six domains and displaying more or less pronounced corrugations parallel to the diagonals, and (C) *semihexagonal* single lamellae with surface features as in A. Since these semihexagons have never been observed in suspension, their formation is thought to occur exclusively during the evaporation process. Other types of morphologies frequently obtained were thicker hexagonal crystals with a coarse surface sometimes showing irregular sectors. In some preparations, fingerlike projections appeared on the edges of the crystals, and eventually a mesh of filaments covered them. Such projections and filaments probably arise from a second crystallization process taking place on the hexagons in such cases. All these variations in crystallinity are most likely influenced by differences in the degree of chain folding and also by segregation according to molecular weight.

A close inspection of the shadow of the three kinds of crystals seems to indicate the presence of a bilayered structure, which is most apparent in the central edge of the semihexagons. Since edges appear poorly defined, an accurate measurement of the lamellar thickness can not be made, but a figure within 150 and 250 Å can be approximatly estimated for the height of these lamellae. This value is about half of the length of a single average helix.

Electron diffraction diagrams containing the 11.7-Å reflection characteristic of the hexagonal form were obtained from these crystals whichever type was examined. However, the resolution displayed by the pattern largely varied from one crystal to another. High-resolution hexagonal diagrams with 6/*mmm* symmetry demonstrative of a well-preserved structure were frequently recorded when crystals of type A or C were selected to diffract (Figure 3D). Poorly defined diagrams with only six spots were instead obtained from crystals of type B, which were presumably distorted during sedimentation and drying. On the other hand, the morphology exhibited by the diffraction pattern depends on the size of the area which is selected to diffract. A pattern consisting essentially of a row of *h*00 reflections was produced when the diffracting area was confined to a small region of type B crystals. Furthermore, it was found that these unidimensional diagrams were oriented with the *h*00 row normal either to the edges or to the diagonals of the hexagon (Figure 4). Such observations may be accounted for if chains are assumed to be tilted, so that the *h*00 series of reflections indicates the presence of parallel planes of tilted molecules. The angle of tilt of the molecules in each plane may vary throughout the whole crystal.

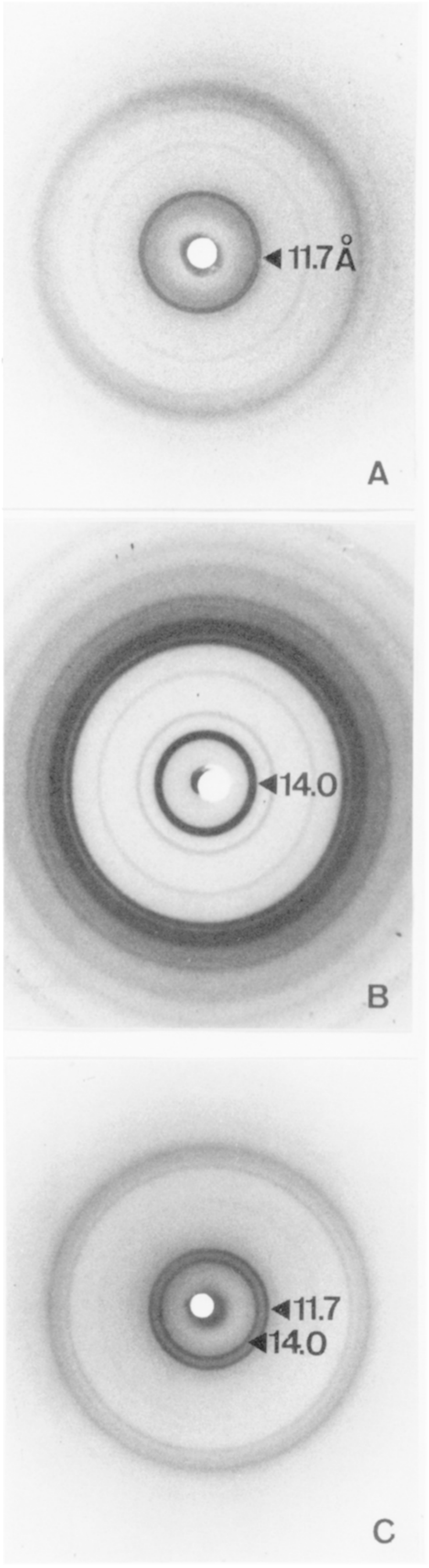

Figure 2. Powder X-ray diagrams of films of PAIBLA cast in (A) chloroform (hexagonal form), (B) 2-chloroethanol (tetragonal form), and (C) trifluorethanol (mixture of both forms). An amorphous halo at about 14 Å is clearly apparent in A.

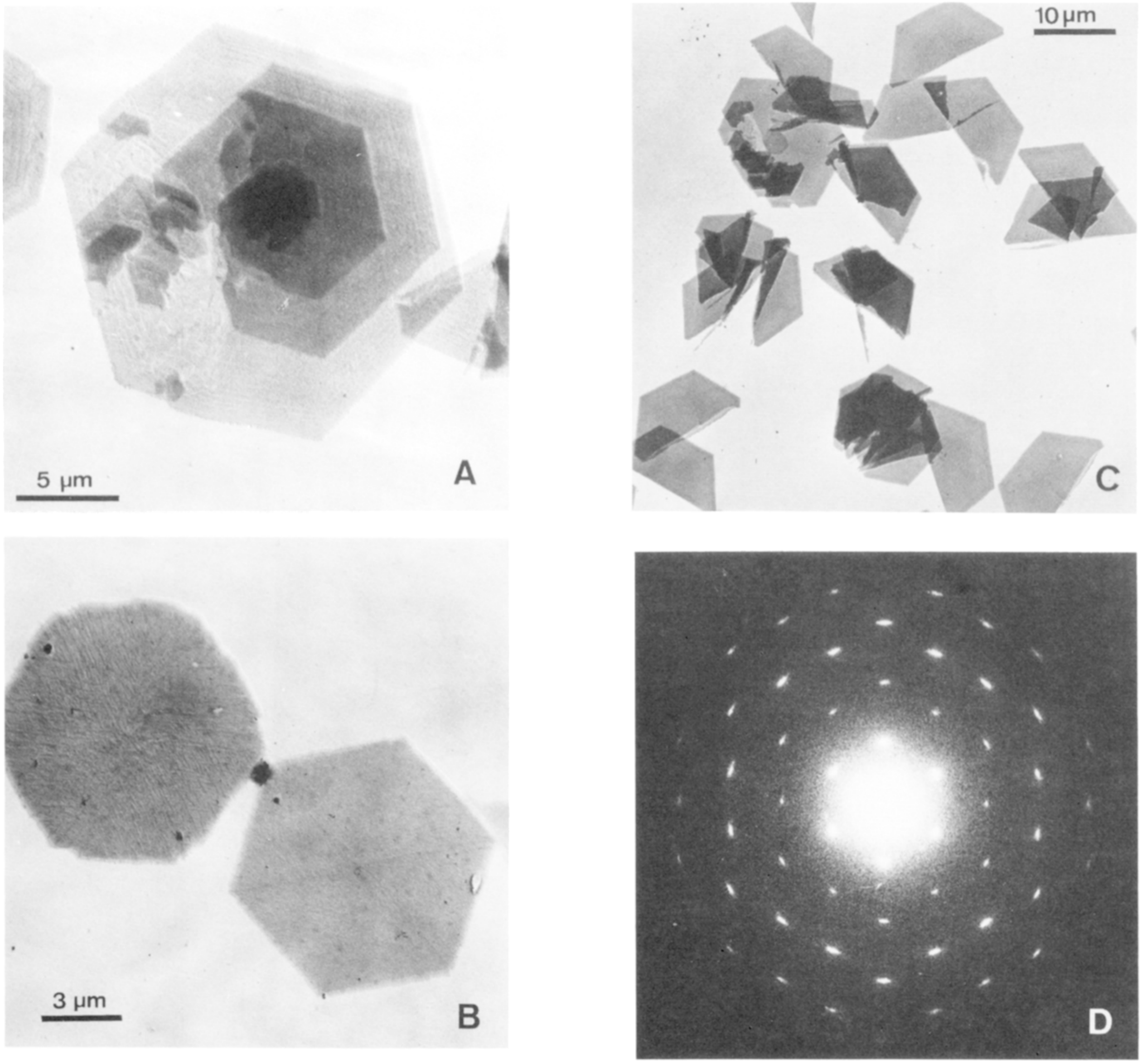

Figure 3. Representative morphologies observed for PAIBLA when crystallized in the hexagonal form: (A) overgrown lamellae; (B) sectorized hexagons; (C) semihexagons; (D) electron diffraction diagram recorded from one hexagonal crystal of PAIBLA, as shown in A. The hexagonal pattern corresponds to a lattice with $a = 13.5$ Å.

Tetragonal Form. Thin films of PAIBLA obtained by stroking a concentrated solution of the polymer in 2-chloroethanol gave diagrams corresponding to the tetragonal form but showed only a faint orientation. On the other hand, crystallization from solution failed to yield this crystalline form, despite the fact that a large number of solvents were tried. Nevertheless, small overgrowth crystals with an approximate square shape (Figure 5) were occasionally found in the preparations of the hexagonal form which had been obtained by crystallization from chloroform–ethanol as described above. Such crystals were presumably formed under the high concentration of ethanol attained in the mixture in the last stages of evaporation. Although only poor diagrams could be recorded from such small crystals, we could discern two pairs of reflections with a common spacing at 14.0 Å and oriented at 90°. This diagram, although faint, conclusively demonstrates the presence of the tetragonal form.

On the other hand, the two forms, hexagonal and tetragonal, were found together as separate entities in samples prepared by evaporation of a dilute solution of the polymer in trifluorethanol at room temperature. Large round platelets giving a hexagonal pattern were present together with long striated rods, such as those depicted in Figure 6A. The diffraction pattern recorded from them is interpreted as arising from a stack of lamellae lying on edge. The equatorial reflections correspond to a tetragonal lattice with $a = 14.0$ Å. The spacings and positions of the reflections along the layer lines are consistent with a helix having four residues per turn with a repeat of 4.95 Å.

Heat Treatment. When hexagonal lamellae of PAIBLA attached onto carbon-covered EM grids were heated at 190 °C for a few minutes, very apparent changes were detected in the morphology as well as in the structure of the crystals. Their thickness increased up to about 500 Å, and their surface became uniformly granulated, except that they appear framed all around by a narrow clearer band (Figure 7). It is interesting to note that such a thickness corresponds to the average length of individual helical molecules. Electron diffraction from these crystals in general produced the complex diagram shown in Figure 8A, which is schematically interpreted in Figure 8B. A satisfactory indexing of this diagram can be made if a mixture of hexagonal and tetragonal forms is assumed to be present in the crystal. It should be noted that while a single pattern is given by the hexagonal form, three

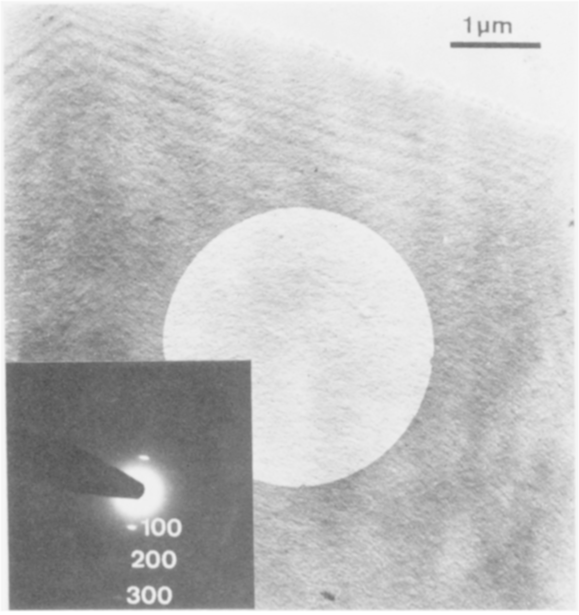

Figure 4. Detail of a hexagonal lamella and the electron diffraction pattern arising from the outlined area. The diagram is placed in corresponding orientation to the crystal in order to show how the *h*00 row aligns normal to the edge of the sector.

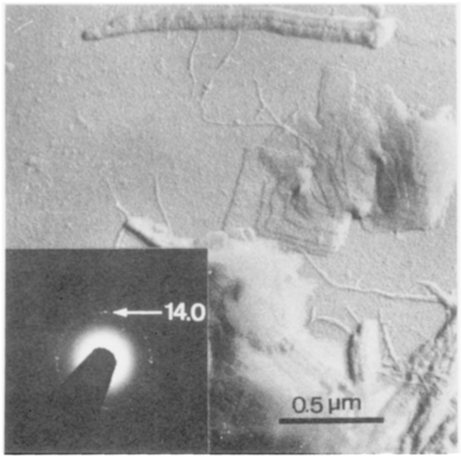

Figure 5. Tetragonal crystals of PAIBLA formed in solution and their corresponding electron diffraction. The two pairs of strongest reflections with a spacing at 14.0 Å are oriented at an angle of 90°, as expected for a tetragonal lattice.

patterns rotated 60° to each other arise from the tetragonal phase. Furthermore, the relative orientation of these patterns reveals that the two forms are in a definite crystallographical relationship; the three tetragonal domains are aligned with their respective 100 axes parallel to the 100, 010, and 110 axes of the hexagonal lattice.

Diagrams with a minor or even no contribution from the hexagonal phase were sometimes observed while grids were scanned for electron diffraction. An example is given in the inset of Figure 8A. They are probably produced by crystals that were locally overheated. In such diagrams, one of the three tetragonal patterns was intensified at the expense of the other two and reflections appeared asymetrically arced, suggesting along what directions domains had been reorganized.

When the thermally induced tetragonal crystals were subsequently heated at 270 °C, they partially melted, and a thinner frame of fingerlike projections of uniform length surrounded the crystals (Figure 9). Electron diffraction from such crystals rendered only poor diagrams with only six reflections hexagonally arranged with an 11.7-Å spacing,

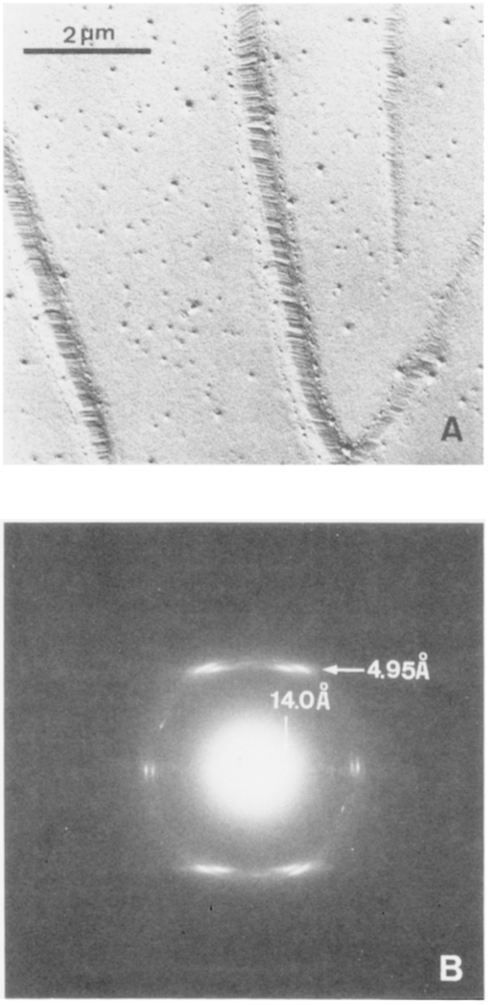

Figure 6. Morphologies originated when PAIBLA is crystallized from a mixture of trifluorethanol–ethanol. Inset: electron diffraction from the lamellar row which corresponds to a tetragonal lattice of a = 14.0 Å and c = 4.95 Å.

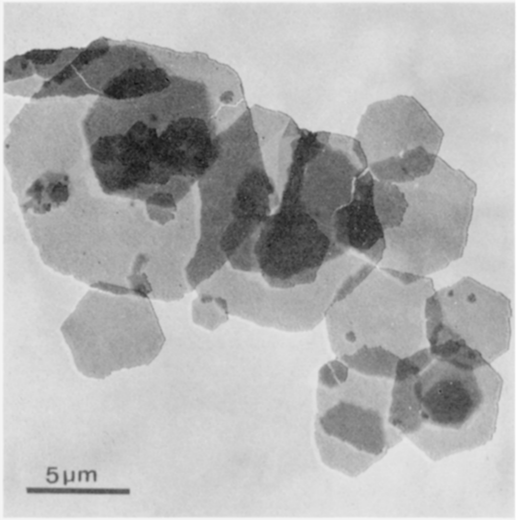

Figure 7. Hexagonal crystals of PAIBLA after heating at 195 °C for 5 min.

which reveals that an imperfect hexagonal packing is adopted by the polymer.

Discussion

It is apparent from this study that the structure adopted by solid samples of PAIBLA grown from solution is largely

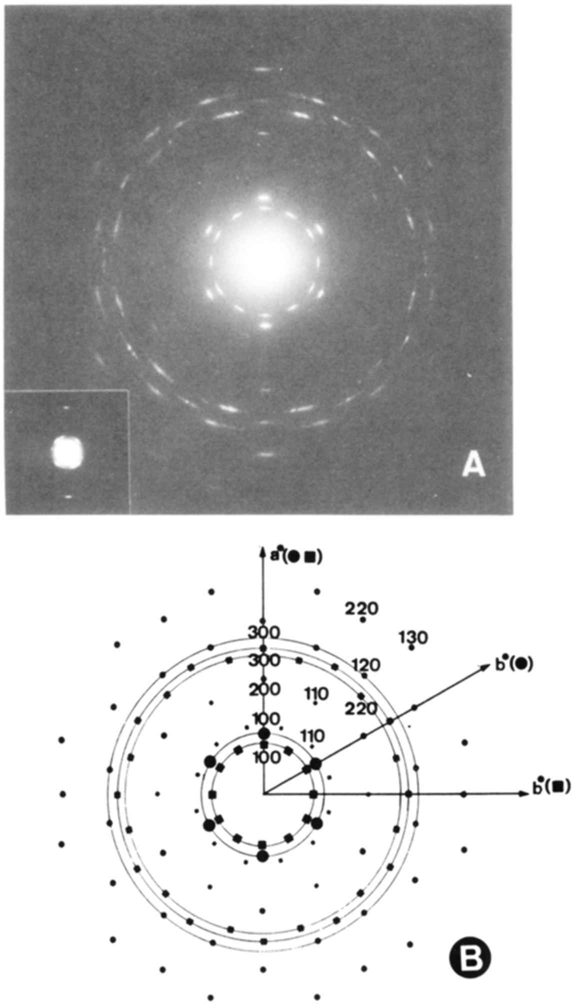

Figure 8. (A) Electron diffraction from one of the lamellae shown in Figure 7 containing both crystalline modifications. Inset: electron diffraction taken from another crystal where the structure has been converted almost completely into the tetragonal form. (B) Scheme including all the reflections seen in the original picture. Indexing has been made on the basis of a hexagonal lattice with $a = 13.5$ Å and a tetragonal lattice with $a = 14.0$ Å. Squares and circles stand for tetragonal and hexagonal, respectively.

decided by the properties of the solvent used, as shown in Figure 2. Whereas helicogenic solvents promote the hexagonal form, hydrogen-bond-associated solvents favor the development of the tetragonal modification. The two forms are simultaneously generated when solvents with both properties are used, as is the case for trifluoroethanol. The effect of ethanol is also worthy of mention: a conversion into the tetragonal form takes place when either single crystals or films in hexagonal form are put in contact with this solvent. Such decisive influence makes the crystallization procedure particularly delicate. Crystal growth can only proceed if a minimum amount of ethanol is present in the crystallization mixture, but at the same time a small excess of this solvent provokes an irregular precipitation of the polymer in the tetragonal form.

Single crystals of polymers having rigid conformations, as is the case for α-polypeptides, are scarcely found in the literature. Sterical hindrance due to chain folding is regarded as the major restriction to the formation of thin lamellae. Nevertheless, several examples of α-helix folded lamellae have been reported, amongst which polyglutamates stand for the case closest to PAIBLA. Hexagonal lamellae very resemblant to those described here have been observed for poly(γ-benzyl L-glutamate)[5] and poly(γ-methyl L-glutamate).[6] Semihexagons were in addition observed in the former case.[7]

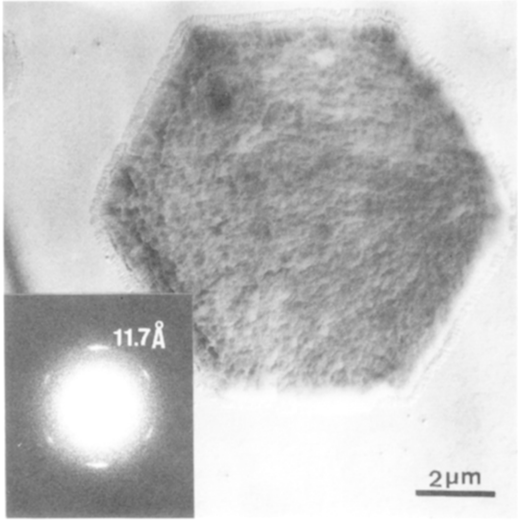

Figure 9. Crystal heated at 270 °C and its corresponding electron diffraction diagram.

Crystals of PAIBLA are made of rigid helices with features similar to the α-helix. The average length of these helices is about 500 Å for the samples used here. Althought this figure is about twice the hexagonal crystal thickness, occurrence of chain folding cannot be straightforwardly concluded, since tilting of chains as well as the complex constitution of the crystals does not allow determination of the actual length of the stem in the crystalline core of the lamellae. Nevertheless, as pointed out by Keller,[8] sectorization must be considered as convincing evidence for folding. Consequently, adjacent reentry should be assumed to happen, at least in sectorized hexagons of PAIBLA. An antiparallel arrangement would result for the chains in such crystals, which is in fact the structural model proposed for the hexagonal form of PAIBLA according to X-ray data obtained from fibers.[2] On the other hand, the bilayered organization observed may be related to the amorphous layer, which must be present in some crystals as a result of the presence of variable lenghts of loose ends of molecules or even of unorganized folds.

We have also observed chain tilting in some cases. This feature is a fact frequently observed in sedimented single crystals of polymers and is usually related to the existence of a hollow pyramidal morphology.[9] A rotation at as much as 70° to their original direction has been observed for the α-helices in distorted crystals of poly(L-lysine).[10] It seems that hexagonal crystals of PAIBLA tend to behave in the same manner, although the tilting angle is smaller and variable in this case since no meridional reflections are recorded in the corresponding electron diffraction diagrams. Our data from individual sectors of the hexagons reveal that, as in poly(L-lysine), reorientation of chains must imply a cooperative motion of mosaic blocks along certain definite directions rather than an homogeneous deformation of whole sectors.

A conversion from the hexagonal to the tetragonal form takes place when crystals are heated at temperatures around 200 °C. Such a transition implies displacements of the helices across the basal plane of the crystal as well as a rearrangement of the molecular conformation. It should be noted that a hexagonal lattice can be readily converted into a tetragonal lattice if parallel planes containing rows of adjacent chains are shifted one-half a helix diameter (Figure 10). Our results strongly indicate that such a mechanism is probably operating in the transition

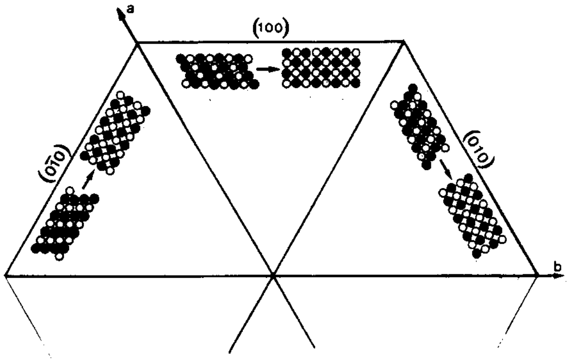

Figure 10. Scheme showing how three tetragonal domains rotated 60° to each other are generated by sliding of planes from a hexagonal single phase.

observed for PAIBLA. In fact, a similar reverse process involving the generation of a twinned hexagonal crystal from a tetragonal single phase was observed by Holland and Miller in isotactic polybutene-1.[11]

During the transition which converts the 13/4 helix of the hexagonal form into the 4/1 helix, a conformational change is also involved. Hydrogen bonds have to be broken and remade, but the detailed molecular mechanism involved is not known since several models are possible for each form of PAIBLA, as discussed elsewhere.[2] It should be noted that the tetragonal crystals obtained upon heating (Figure 7) appear to be thicker than the hexagonal crystals from which they originate, but it is not clear through what mechanism this result is achieved.

A hexagonal packing with an equatorial spacing of 11.7 Å is found when PAIBLA is heated at temperatures above 250 °C. This structure should not be identified with the hexagonal structure present in the crystals prepared from chloroform solution. In any case, it is much more disodered, as shown by its electron diffraction pattern. They may differ either in the main chain conformation or in the lateral organization of the helices. A nematic structure of hexagonally packed chains with the side groups filling a disordered interchain region would be an appropriate model consistent with both diffraction data and the thermal history of the sample. However, any interpretation of the structure generated at these high temperatures should be made with caution, as some decomposition of the polymer likely occurs under such conditions. In fact, a significant amount of imides is detected in these heated samples by infrared spectroscopy.

Acknowledgment. We are indebted to the Francisca María Roviralta Foundation for the donation of the electron microscope used for this work. Financial support for this research was obtained from the Comisión Asesora de Investigación Científica y Técnica (Grant No. PR-84-0161) and from the industrial Spanish firm ERT (Explosivos Riotinto). C.A. acknowledges a predoctoral FPI fellowship.

Registry No. PAIBLA (homopolymer), 35239-25-9; PAIBLA (SRU), 37768-91-5.

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