monomeric glasses that Δh^* and x do not. The differences are such that comparable values of D correspond to larger values of T_g/T_2 for the inorganic/monomeric glasses. We speculate that, to the extent that D is a good measure of $\Delta\mu$, these differences may reflect three-dimensional configurational constraints for the inorganic/monomeric glasses that prevent them from approaching T_2 as closely as polymers, whose constraints are closer to being one-dimensional and may therefore be less restrictive.

Applications of eq 6 to experimental data, investigation of other functional forms generated from different assumed temperature dependences of $C_{p,c}$, and tests of eq 8-11 are in progress. The results of these studies, together with a more detailed consideration of the AGF formalism and its implications, will be reported elsewhere.[17]

Acknowledgment. I thank G. W. Scherer for stimulating discussions and preprints of ref 14.

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Received October 2, 1985

Spherulites of Poly(L-histidine hydrochloride)

A histidine residue is an essentil constituent of the active site of several enzymes and plays a significant role in enzymic reaction, and it is also a ligand in some important metalloproteins.[1] Therefore the catalytic action of poly(L-histidine) (PLH) and its ligand characteristics have been the subject of intensive studies as a model for proteins with histidyl groups.[2] The interaction of PLH with DNA has also been examined.[3] Recently histidine-rich protein (HRP) has been found in the intraerythrocytic stages of development of the malaria parasite *Plasmodium lophurae* in ducks. Kilejian reported that HRP acts as an antigen and suggested the possibility of synthesizing malaria vaccine based on HRP,[4] though others have challenged this observation.[5] HRP comprises 73% histidine, and the histidine residues are incorporated almost exclusively as pentamers to nonamers in tandemly repeated units. The four major amino acid residues other than histidine (Ala, Glu, Pro, and Asp) link oligohistidines.[6] Thus PLH is regarded as a significant structural model of HRP as well.

Despite extensive investigations, no definite consensus seems to have been drawn as to the structure of PLH in aqueous alkaline solution,[7] while a recent Raman examination has led to the conclusion that PLH ($\bar{M}_w = 5000$) assumes the β-form in aqueous gel at pH >6.5.[8] On the other hand, it is believed that PLH adopts a random conformation in acidic media.[7] From an infrared study, it was inferred that, in solid films, neutral PLH assumes the α-helical-form, and its hydrochloride presumably a random form.[9]

We have found that poly(L-histidine hydrochloride) crystallizes from aqueous solution in the form of negative spherulites large enough for an X-ray diffraction study. Here we propose that the left-handed 15/4 helix is a plausible structure of PLH hydrochloride based on its X-ray fiber photograph, its IR spectrum, and conformational energy calculation.

PLH[10] ($\bar{M}_n = 1.4 \times 10^4$, $\bar{M}_w/\bar{M}_n = 1.43$[11]) was dissolved in a slight excess of 1 N HCl, and the solution was lyophilized to yield white chips which contained 1 equiv of Cl^- and 1 mol of hydration water per residue as in the literature.[10] One milligram of the hydrochloride was placed on a glass slide (18 × 18 mm), 0.2 mL of distilled water was added to give a clear solution, and the mixture was spread over the slide. The solution was covered with a petri dish (2 × 10 cm) with a small vessel containing ca. 1 mL of water beside the slide and allowed to stand at room temperature (ca. 25 °C). After several days, the surface of the slide became opaque due to the growth of spherulites. For X-ray diffraction measurements, crystals developed on the glass slide were peeled off by the use of a sharp blade. A piece of the spherulite (10 × 500 μm) was attached at the tip of a steel needle and held in front of a collimeter (diameter, 100 μm). A micro flat-plate camera was used to take an X-ray diffraction photograph with an unfiltered microbeam of X-rays from Cu generated through a Rotaflex instrument (Rigaku Corp., Tokyo) at 45 kV and 200 mA.

In a photograph of the spherulite taken between crossed polaroids, a Maltese cross was observed (Figure 1). The spherulites showed negative birefringence, implying the molecular axis to lie in the tangential direction. The spherulites (KBr disk) showed an amide I IR band at 1653 cm^{-1} with a shoulder around 1680 cm^{-1} and an amide II band at 1527 cm^{-1}. This profile was same as that obtained from the film[9] and differed from those of either the α-helix or the β-form.[12] Further an amide I Raman line of spherulites appeared at 1686 cm^{-1},[13] which was also explicitly different from those of the α-helix, the β-form, and even the random coil.[14]

An X-ray fiber pattern was obtained by irradiating an X-ray beam normal to the surface at the circumference (Figure 2). The Bragg spacings of this diffraction pattern were indexed to a quasi-hexagonal lattice with $a = b = 14.95$ Å, $c = 45.0$ Å, and $\gamma = 116.5°$ (Table I) using Mitsui's graphical method.[15] The c axis lies tangential to the spherulite radius. The density was calculated to be 1.55 g cm^{-3} assuming that a unit cell contains three polymer chains, each residue of which is associated with 1 equiv of Cl^- and 1 mol of hydration water. This is not inconsistent with the observed density (1.44 g cm^{-3}).

The conformation of the polymer is a 15/4 helix with a monomer repeat of 3.00 Å. On the basis of standard values of bond lengths and bond angles of the main-chain

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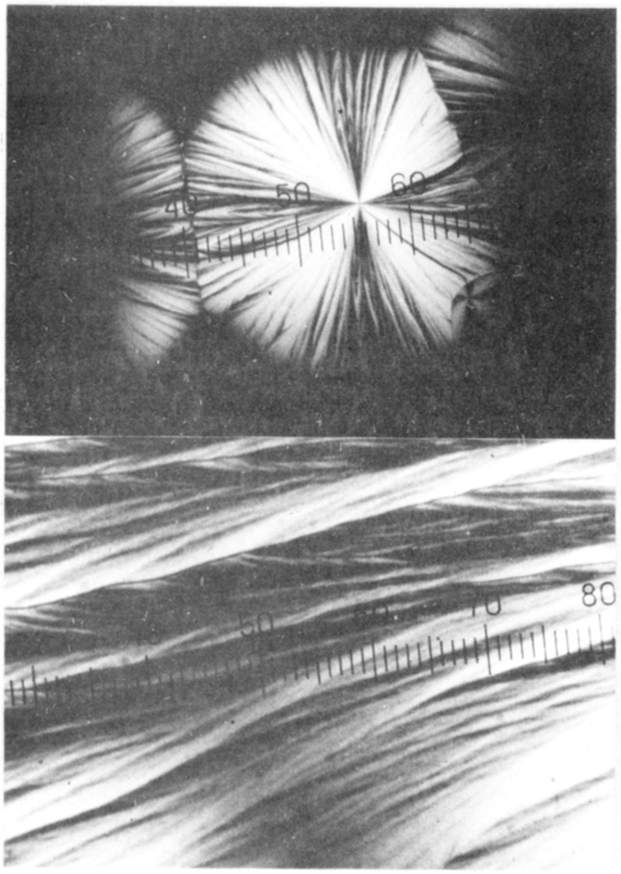

Figure 1. Photograph of spherulite of poly(L-histidine hydrochloride) between crossed polaroids; 27.3 (top) and 2.73 μm/div (bottom).

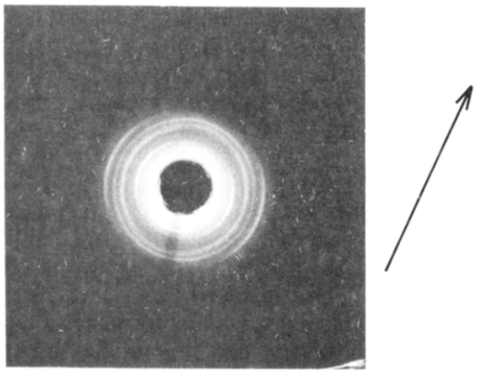

Figure 2. X-ray fiber pattern at the circumference of the spherulite of poly(L-istidine hydrochloride) obtained by irradiating an unfiltered X-ray beam from Cu normal to the surface. The arrow indicates the tangential direction of the spherulites.

group,[16] the respective values of φ and ψ were determined to be −72.01° and +173.65° by Newton's method. These values represent the left-handed helix; the corresponding right-handed helix was of extremely high energy as revealed by an intramolecular potential energy calculation and was readily excluded.

The intramolecular conformational energy contours of the central residue of nona-L-histidinium was estimated[17] by varying the (χ_1, χ_2) values at a fixed (φ, ψ) value of (−72.01°, +173.65°) (Figure 3). Although the rather flat, low-energy wells are given when χ_1 is set at 300°, the energy minimum resides at (χ_1, χ_2) = (80°, 145°). It is noticed that this χ_1 value is close to that in the crystal of L-histidine hydrochloride monohydrate (71.5°),[20] though the χ_2 value differs by about 90°. This (χ_1, χ_2) value was also predicted to be possible for the cationic form of L-alanyl-L-histidine diketopiperazine in solution.[22]

Table I
Observed and Calculated Spacings of X-ray Reflections from the Spherulites of Poly(L-histidine hydrochloride)[a]

hkl	d_{obsd}, Å	k_{calcd}, Å	intens
$l = 0$ ($n^b = 0$)			
110	7.85	7.85	m
200	6.59	6.59	m
210	5.20	5.16	s
400	3.35	3.32	w
320	3.10	3.05	w
410	2.93	2.90	w
420	2.59	2.58	w
510	2.40	2.40	w
$l = 4$ ($n = 1$)			
304	4.14	4.11	m
$l = 7$ ($n = -2$)			
107	5.90	5.88	m
$l = 11$ ($n = -1$)			
1,0,11	3.90	3.90	m
1,1,11	3.65	3.65	m

[a] Monoclinic, $a = b = 14.97$ Å, $c = 45.0$ Å, $\gamma = 116.5°$. [b] Order of the Bessel function.

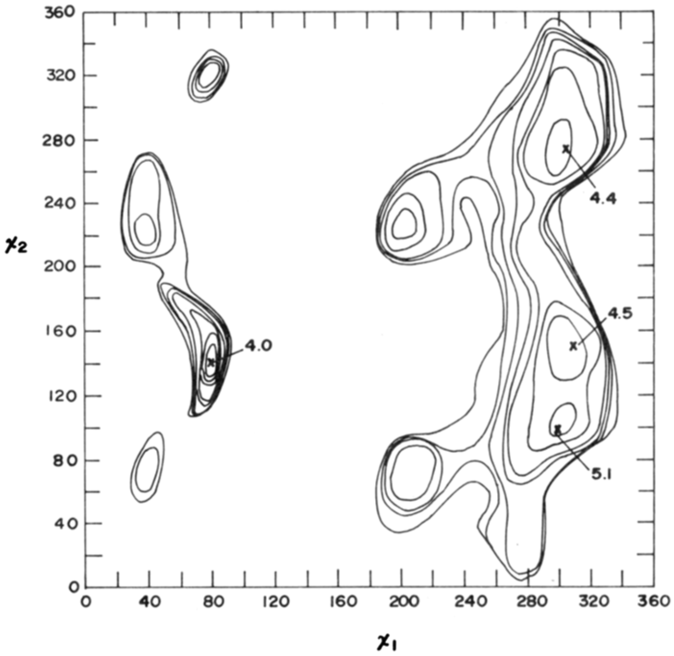

Figure 3. Potential energy contours of the side-chain conformation of the left-handed 15/4 helix of poly(L-histidinium) with (φ, ψ) = (−70.5°, +171.5°). Contours are spaced at intervals of 1 kcal/mol residue. Letters in kcal/mol residue.

The more significant fact is that the above (χ_1, χ_2) value provides a considerably compact side-chain conformation as compared with that with χ_1 = 300°, and then it is easy to pack three polymer chains in the unit cell (Figure 4a).[23] In Figure 4a the relative displacement along the helix axis and relative rotation of the neighboring helices are 1.5 Å and 92°, respectively, where no overly short contact is observed. This finding may advocate the proposition that the 15/4 helix is a good candidate for the structure of the polymer.

In the 15/4 helix, the planar trans peptide group is inclined at ca. 35° to the helix axis. The C═O group locates near the axis or at the inner part whereas the N–H group is at the outer part (Figure 4b), which is similar to the threefold helix[24] and the fourfold helix[25] proposed for polyglycine (form II), polyproline, and copolymers of glycine and proline. This location seems consistent with the conclusion deduced from the spectroscopic data that C═O makes rather weak hydrogen bonding and N-H strong

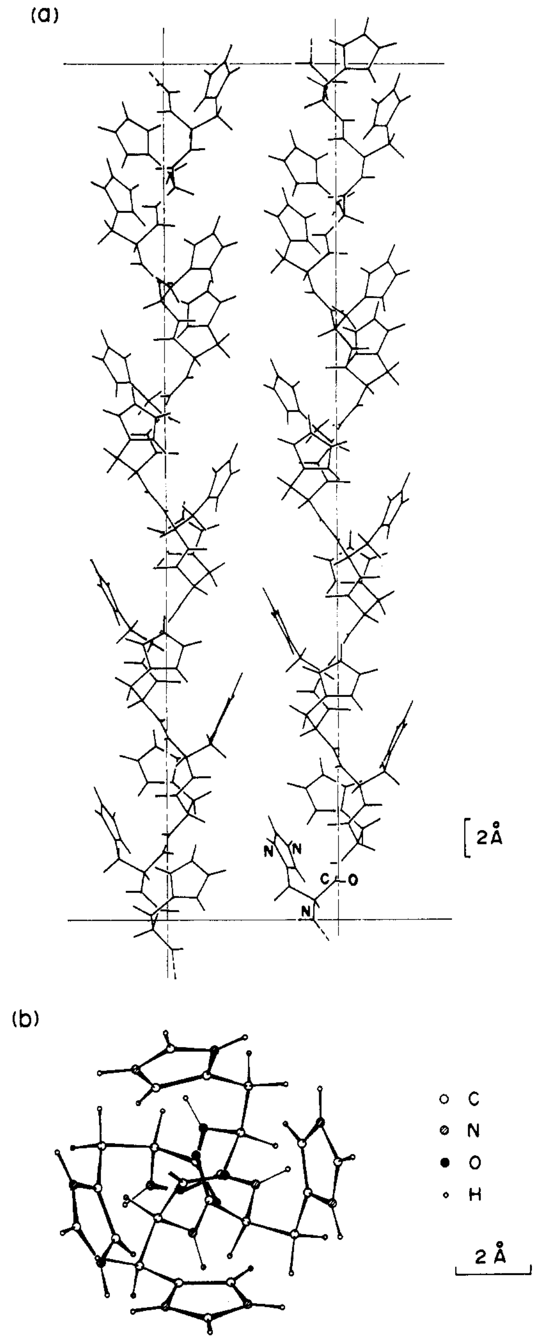

Figure 4. Left-handed 15/4 helix of poly(L-histidinium): (a) projection of repeating units of neighboring molecules onto a [110] plane; (b) projection of first four residues down a helix axis.

hydrogen bonding, probably with Cl^-.[9] Hydration water and Cl^- ion may contribute to stabilize the structure of PLH hydrochloride through the formation of hydrogen bonding with imidazolium and amide N-H groups, and further they should affect the X-ray diffraction intensities to a great extent. The determination of their definite positions, however, requires more refined investigation.

In summary, poly(L-histidine hydrochloride) was found to crystallize in the form of spherulites and the IR and Raman spectra differed from those of conventional α, β, and random forms. The left-handed 15/4 helix was proposed as a plausible conformation of the polymer in spherulites.

Acknowledgment. We thank Prof. V. Sasisekharan for critical comments and H. Maruyama and S. Kumazawa of Rigaku Corp. Ltd. for taking the X-ray diffraction photographs.

Registry No. Poly(L-histidine hydrochloride) (homopolymer), 61857-39-4; poly(L-histidine hydrochloride) (SRU), 70605-42-4.

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(17) The intramolecular potential energy was calculated as a sum of nonbonded interaction and torsional energies for rotation about C^α–C^β (χ_1) and C^β–C^γ (χ_2). The Lennard-Jones 6-12 potential was used to calculate the energy between a pair of unbonded atoms with parameters given by Momany et al.[18] The rotational barriers for χ_1 and χ_2 were the same as used in the previous paper.[19] Standard values of bond angles and bond lengths were applied for the main-chain group[16] and the geometry of the imidazolium group was that of the crystal of L-histidine hydrochloride monohydrate.[20] Regions at $\chi_1 = 300$ became very favorable when the pairwise electrostatic interaction energy was added to the contours, which was estimated by Coulomb's law using partial charges on the respective atoms given by CNDO/2 calculation[19] and the tentative dielectric constant of 4,[21] where shielding effects of counteranions were ignored. However, calculation of the electrostatic energy is troublesome, especially for the ionic crystal in which permanent charges are placed periodically. So we here considered the energy contours without electrostatic interaction. This assumption is rationalized at least for evaluation of the conformational energy to eliminate impossible conformations.

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Received September 12, 1985

Three-Dimensional Order in Magnetically Oriented Poly(γ-benzyl L-glutamate) Films

Orientation of rodlike polymers using strong magnetic fields has been investigated for quite some time.[1-8] The diamagnetic anisotropy of such polymers is small, and as a consequence the magnetic orientational energy is not sufficient to orient individual molecules. However, if the molecules aggregate into an ordered array (e.g., virus particles[5,6] or fibrils[7,8]) or form an ordered fluid phase (liquid crystalline phase[1-3]), the diamagnetic anisotropy is enhanced considerably and highly oriented films can be easily obtained with magnetic fields of strength 0.2–2 T. Oriented DNA-containing bacteriophage was obtained by suspending a 2% solution of the virus over a 2-mm gap formed between horizontal glass rods: a magnetic field was used to enhance the uniaxial orientation induced mechanically by the glass rods during the slow evaporation of the solvent.[5,6] Experiments with oriented fibrin gels[7] obtained by polymerization in a magnetic field have shown that although the fibrin monomers (length 450 Å) are arranged in a crystalline lattice within a fiber (diameter ca. 1000 Å), the gel (network of fibers) as such is rotationally symmetrical about the molecular axis (direction of the magnetic field) with little long-range rotational order between the fibers. In the optical microscopy work reported on magnetically oriented collagen reconstituted from rat tail tendon,[8] no effort was made to determine the presence or absence of rotational symmetry around the orientation direction.

In one of the earliest works on magnetic orientation of polymers,[2] it was concluded that α-helical poly(γ-benzyl L-glutamate) (PBLG) films exhibit uniaxial orientation similar to mechanically oriented[9] polypeptide fibers. We now show that magnetically oriented PBLG has a symmetry higher than that of uniaxially oriented fibers. Unlike fibrin gels[7], the magnetically oriented PBLG film is not rotationally symmetric but possesses three-dimensional order extending over the entire film (0.5 cm × 1 cm and 0.1–0.5 mm thick) to a resolution of at least 2.6 Å.

Homogeneous solid films of high molecular weight PBLG (Sigma Chemical Co., stock no. P-5136, lot 074F5082, MW $\simeq$ 150 000–300 000) were prepared by slowly evaporating solutions of PBLG in CH_2Cl_2 over a period of ~24 h. Oriented films (thickness of 0.1–0.5 mm) were obtained when the solutions were evaporated in a 2-T magnetic field with the field direction parallel to the casting surface. A control film was made under similar conditions but in the absence of a magnetic field. A second control specimen was prepared by drawing a fiber from a concentrated solution of PBLG in CH_2Cl_2. X-ray diffraction (XRD) photographs were obtained at room temperature on a flat film by using a Unicam camera or a Supper precession camera with Ni-filtered Cu radiation (sample-to-film distance of 6.0 cm).

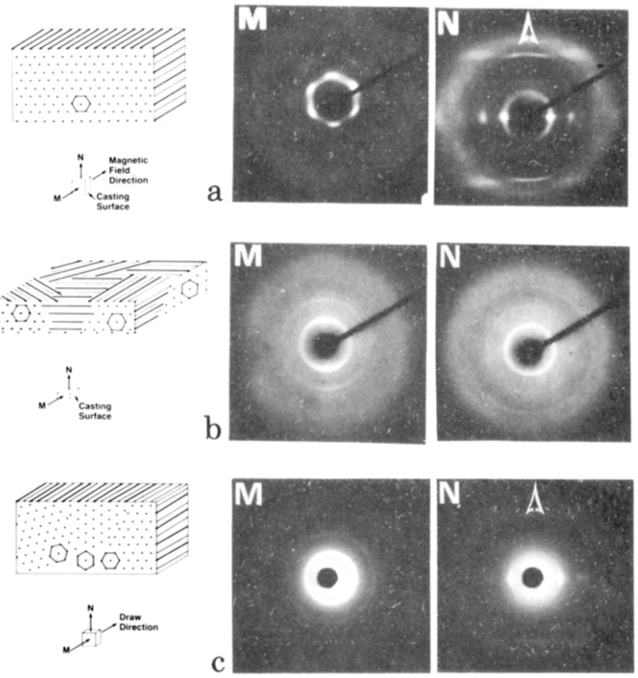

Figure 1. X-ray diffraction photographs of poly(γ-benzyl L-glutamate) (PBLG) samples. Such patterns can be indexed on a hexagonal lattice of side 14.8 Å. (a) Magnetically oriented film; (b) film prepared in the absence of a magnetic field but otherwise under conditions similar to those in (a); (c) fiber drawn from a concentrated PBLG solution. Left-hand side photographs, labeled M, were obtained with the film horizontal and with X-rays along the "machine" direction (respectively, direction of the field in (a), a direction parallel to the surface of the film in (b), and the direction of drawing in (c)). Right-hand side photographs, labeled N, were obtained with the film vertical and with X-rays normal to the surface of the film ((a) and (b)) or to the draw direction (c). The orientation of the sample with respect to the X-ray beam for the M and N photographs is illustrated next to each set of photographs. The direction of the magnetic field or drawining is also shown in these diagrams as well as in (a) and (c). The photographs of the magnetically oriented film obtained with X-rays perpendicular to the direction of the field and to the film normal are similar to the N photograph of (a). This similarity arises from the overlapping of the equatorial reflections.[10] (a) Shows three-dimensional order up to 2.6-Å resolution, (b) shows planar order with the crystallites of PBLG molecules randomly oriented in the plane of the film, and (c) shows uniaxial orientation with PBLG molecules oriented along the draw direction. These three states of order are schematically shown adjacent to each of the figures.

Figure 1a shows typical XRD photographs obtained with X-rays along the direction of the magnetic field (M direction) and along the normal to the casting surface (N direction).[14] For comparison, photographs from films made under identical conditions but with no magnetic field are shown in Figure 1b, and photographs of a fiber drawn from a concentrated solution are given in Figure 1c. In the absence of the field, there is no preferred orientation within the plane of the film (Figure 1b,N). While the N photograph of the magnetically oriented film (Figure 1a,N) shows a diffraction pattern similar to that of a uniaxially drawn fiber (Figure 1c,N), the M photograph (Figure 1a,M) shows a hexagonal array of reciprocal lattice points when one looks down the chain axis, i.e., the direction of the magnetic field. This hexagonal pattern persists up to a Bragg spacing of at least 2.6 Å, and the pattern from any region of the 0.5 cm × 1 cm magnetically oriented film appears in this same orientation. This indicates that the rotational disorder of the mosaic blocks common to cast films (Figure 1b,N) and uniaxially drawn fibers (Figure 1c,M) is absent and that in magnetically oriented PBLG films the crystalline domains or mosaic blocks show

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