SOLID 'LIQUID-CRYSTALLINE' FILMS OF SYNTHETIC POLYPEPTIDES: A NEW STATE OF MATTER

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ABSTRACT

Concentrated solutions of poly-γ-benzyl-L-glutamate (PBLG), while still in a fluid condition, have previously been shown to be in a liquid crystalline state, characterized as helicoidal or 'cholesteric'. We have found that this same structure persists in certain *solid* films of PBLG. This conclusion was reached from studies of swelling, x-ray, NMR and optical properties. Orientation of the PBLG molecules could be achieved by casting films in a strong magnetic field. A new helical conformation for PBLG was observed in oriented films of this type which were cast from chloroform.

The liquid crystal state for low molecular weight compounds such as cholesterol esters has been known for more than seventy-five years. This is a mesomorphic state of matter, intermediate between the crystal state and the liquid state. In the liquid crystalline state the molecules retain preferential orientations relative to one another over large distances—a property normally associated with the crystalline state; yet liquid crystals from low molecular weight substances are highly fluid in their thermodynamically stable condition.

Recently liquid crystals have received much attention because of their optical properties which enable one to monitor minute changes in temperature and mechanical stress, for their ability to orient in electric and magnetic fields, and because of their implications on structures observed in biological systems.

In the 1930's it was shown by Vorlander[10] that some liquid crystals could be quick frozen to a metastable brittle glassy state. By working with a polymeric molecule, a synthetic polypeptide called poly-γ-benzyl-L-glutamate, we have been able to obtain stable solid films with a liquid crystalline local structure. These films can be obtained in conditions describable as rubbery, leathery, or glassy, as is common for polymer films. The unusual local structure of the molecules in the liquid crystalline phase gives rise to magnetic and optical properties not previously obtainable with polymeric systems.

Fifteen years ago it was observed by Doty *et al.*[1] that synthetic polypeptides, $-(-NH-CHR-CO-)_n-$, in solution can exist in a rigid rodlike α-helical conformation, in contrast with the random coil shape assumed by most other synthetic polymers in solution. The observation has stimulated a large body of investigation of the dilute solution properties of this class of polymers. In more concentrated solutions (in the range of ten to fifty per cent polymer), Robinson[2] found that poly-γ-benzyl-L-glutamate

(PBLG; R = CH_2CH_2CO—O—$CH_2C_6H_5$), a readily available synthetic polypeptide, forms a lyotropic liquid crystalline phase. This means that locally, the PBLG molecules are arranged relative to one another in a very specific manner and this order extends to macroscopic dimensions throughout the solution. The liquid crystalline phase exists for melts of pure substances (thermotropic liquid crystals) as well as for solutions (lyotropic liquid crystals). The solutions that Robinson studied were quite fluid, as are the liquid crystal phases of smaller molecules whether thermotropic or lyotropic. The molecular arrangement in the liquid crystalline phase of PBLG is similar to the helicoidal structure found in the liquid crystalline phase of many pure cholesterol esters and is easily recognized with a polarizing microscope. We shall refer to this type of structure found by Robinson as 'cholesteric'. When viewed between crossed polars these birefringent solutions present an image very reminescent of a fingerprint. The spacing between the alternating bright and dark retardation lines is equal to one-half of the pitch of the 'cholesteric' structure (*Figure 1*, liquid).

We have gone beyond the work of Robinson by casting *solid* films from various solvents and mixed solvents. These solid films are either pure PBLG or contain predetermined amounts of non-volatile liquids which act as plasticizers for the film.

Studies of x-ray diffraction patterns, specific volume and mechanical properties of PBLG films led us to infer that some of these solid films cast from certain solvents such as chloroform and methylene chloride retained the local structure of the liquid crystalline phase[3]. On the other hand, conventionally crystalline films of PBLG were obtained from the solvent dimethyl formamide[4].

Consideration of the molecular structures of solvents which promoted the formation of the 'cholesteric' liquid crystalline phase prompted the selection of the non-volatile liquid 3,3′-dimethylbiphenyl as a plasticizer for the films. Using this plasticizer it is possible to obtain solid films even at relatively low concentrations of PBLG (less than twenty per cent polymer). Controlled evaporation of chloroform solutions of PBLG + plasticizer resulted in solid films which retained the optical retardation lines characteristic of the 'cholesteric' structure (*Figure 1*, solid). This evidence is quite convincing in that the unusual supramolecular arrangement of the liquid crystalline phase does exist in the solid state of mixtures of PBLG and plasticizer. When solid 'cholesteric' films (with or without plasticizer) are cast from solvents such as $CHCl_3$ or CH_2Cl_2, x-ray evidence and anisotropic swelling characteristics clearly indicate that the PBLG rods lie in the plane of the film, but with no preferred direction in this plane[6].

The fact that these films are 'solid' with regard to mechanical properties but 'liquid crystalline' in structure is different from the fluidity hitherto associated with liquid crystalline phases. This phenomenon probably results from the high molecular weight of the PBLG molecules.

The nuclear magnetic resonance (NMR) spectra of 'solute' molecules in fluid nematic liquid crystal 'solvents' show additional splittings due to direct dipole–dipole coupling. Three sets of workers (Samulski and Tobolsky[6], Sobajima[7], Panar and Phillips[8]) found independently that in concentrated solutions of PBLG + CH_2Cl_2 the NMR spectrum of CH_2Cl_2 was split

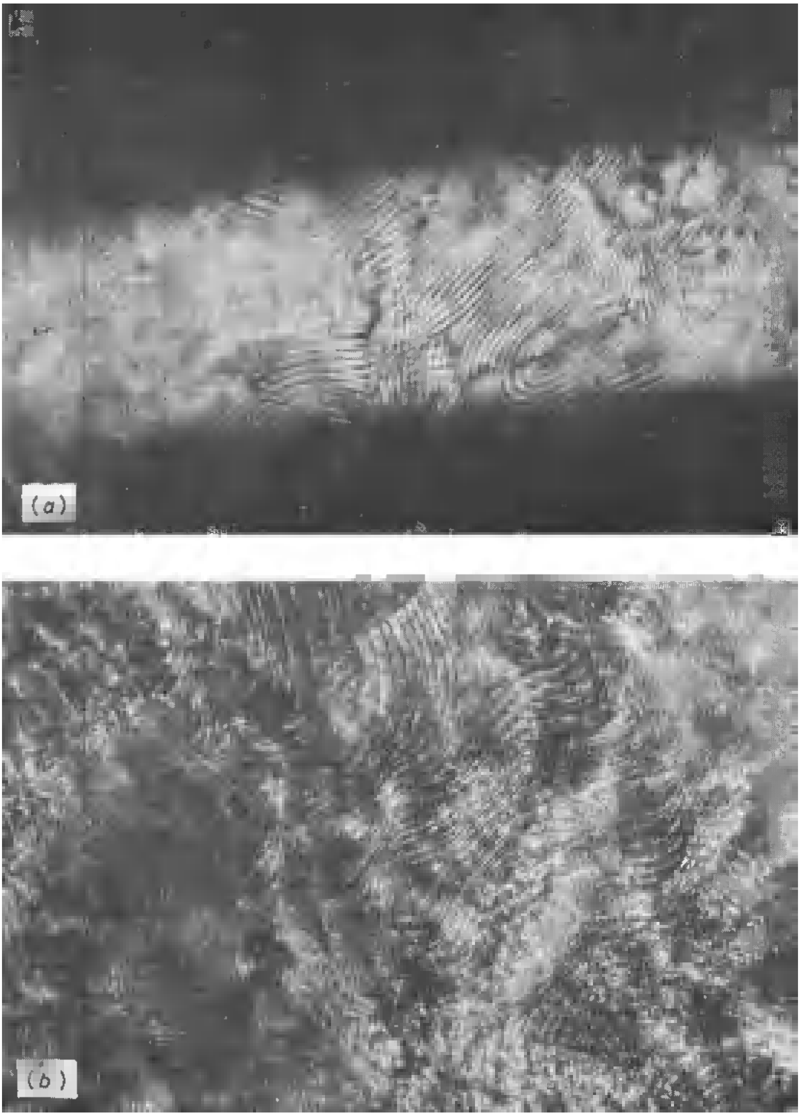

Figure 1. Retardation lines characteristic of a helicoidal supra-molecular structure are observed in the photomicrographs of both the liquid and solid states of poly-γ-benzyl-L-glumatic (PBLC).

(a) Birefringent fluid liquid crystalline solution of PBLG in dioxane, S = 50 microns, 10% PBLG (vol.).

(b) Birefringent solid film of PBLG plasticized by 3,3′-dimethyl biphenyl, S = 2 microns, 30% PBLG (vol.).

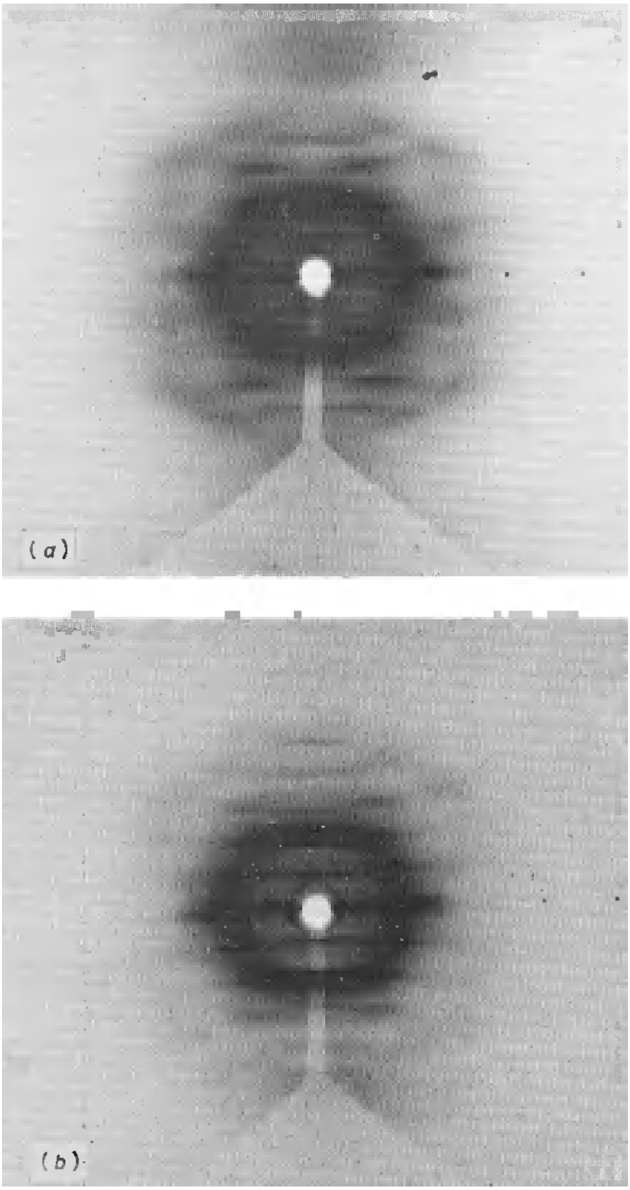

Figure 2. X-ray diffraction patterns of magnetically oriented PBLC films. The photographs were recorded with the fibre axis perpendicular of the cylindrical camera axis and the incident x-ray beam using a 200 micron collimator.

(a) PBLG cast from CH_2Cl_2.
(b) PBLG cast from $CHCl_3$.

into a doublet. It appears that the 'cholesteric' structure of the PBLG solution is untwisted by the magnetic field to form a nematic liquid crystalline structure in which the rodlike molecules are oriented parallel to the field. Splitting of the NMR absorption of CH_2Cl_2 molecules in this kind of environment is to be expected.

We found in addition that cast films of PBLG containing 5 to 20 per cent CH_2Cl_2 (films that are solid) showed splitting of the CH_2Cl_2 absorption. The change in the NMR spectra of the swollen films with the orientation of the film in the field also indicates that the helical molecules in the solid film are randomly oriented in the plane of the film[5].

It has been known for some time that magnetic fields of the order of several hundred oersteds cause spontaneous ordering of liquid crystals. The local order present in the liquid crystalline phase allows the magnetic field to interact with the magnetic anisotropy of a large number of molecules in a cooperative manner; this causes orientation parallel to the field in the direction along which the diamagnetism is the smallest. In fluid liquid crystals the orientation disappears rapidly when the magnetic field is removed.

Samulski and Tobolsky[6] and Sobajima[7] discovered independently that when films of PBLG are cast in the presence of a strong magnetic field they are highly oriented. Orientation occurs while the solutions are in the fluid liquid crystalline phase and becomes permanently locked in when the mixture of solvent plus PBLG becomes solid. We found in fact that the molecules could be oriented in any given direction in the plane of the film, or even perpendicular to the plane of the film[6]. X-ray diffraction patterns from these films are very similar to the fibre patterns obtained from mechanically oriented fibres of PBLG. *Figure 2(a)* shows the diffraction pattern of an oriented film cast from chloroform in a magnetic field of 5,000 oersteds. The strong layer line at 10·4 A, and the absence of other layer lines between the equator and the turn layer line, clearly demonstrates that the helical parameters are 7 residues in 2 turns (3·5 residues per turn) in contrast to the 'normal' α-helix, 18 residues in 5 turns (3·6 residues per turn). The deformation of the 'normal' α-helix to give a conformation with 3·4 residues per turn was postulated earlier by Tsuboi *et al.*[9] to permit sidechain--sidechain interactions between neighbouring helices and thereby account for the diffraction pattern of a 50:50 mixture of D and L polybenzyl glutamate. The 'normal' α-helical diffraction pattern shown in *Figure 2(b)* with 3·6 residues per turn is observed in oriented films cast from methylene chloride. These observations suggest that the small differences in molecular conformation present in different solvents might be detected by utilizing this new technique for orientating synthetic polypeptides.

Solid 'liquid crystal' films of synthetic polypeptides present a new and very interesting phase of matter. It is quite possible that other rodlike molecules which exhibit a liquid crystal phase such as DNA can be induced to form in this same state.

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