Liquid Crystals as They Relate to the Structure of Proteins

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Liquid crystals are discussed from the standpoint of the types of organic molecules which exhibit this state of matter when heated, as well as those systems which show the properties of liquid crystallinity when prepared from two or more components. Information on the structure and properties of liquid crystals as determined from several experimental methods are presented. Considering the structural characteristics of the liquid crystalline state, its potential role in living systems is discussed. Some of the phase transitions in purified proteins are presented as being liquid crystalline, for example, deoxygenated hemoglobin (Hb-SS) of sickle cell anemia and myoglobin. With the demonstration of a distinct liquid crystalline phase in a synthetic polypeptide, *viz.*, poly-α,γ-benzyl-L-glutamate, the possibility of the occurrence of the liquid crystalline state in protein-predominant biologic systems is strongly suggested. Many polypeptide-solvent systems like poly-α,ϵ-carbobenzoxy-L-lysine, poly-α-sodium-L-glutamate, and poly-α,β-benzyl-L-aspartate are examined from this point of view.

The liquid crystalline state is important in living systems. Slight changes in composition and in physical and chemical properties can materially affect the formation, continuation, or cessation of the liquid crystalline state. This is relevant for the delicate balance of state and phases characteristic of many living processes. Catalytic processes in biological systems could thus readily find a favorable and even essential environment in this state. The extended molecules, with fairly strong dipoles and easily polarizable groups so characteristic of biological fluids, are comparable to those molecules of inanimate nature which exhibit liquid crystallinity.

The idea that liquid crystals are intimately involved in living systems is not new. The idea was proposed by Lehmann (1889), one of the original investigators in the field of liquid crystals. The first article on liquid crystals in the English language dealt with the role of liquid crystals in living systems (Lehmann, 1915). The lack of utility and the limited knowledge of the structure and properties of liquid crystals resulted in their disappearance from biological literature until recent years. Many structural characteristics and properties of living systems, such as multiplicity of shapes and a high degree of order at relatively long ranges, are conditions uniquely met by liquid crystals. They allow for liquid-like diffusion and for transformation of energy and information in a selective manner over long distances. Liquid crystals respond to heat changes, to light, sound, mechanical pressure, and chemical environment with a sensitivity not often found in other states of matter. The role of liquid crystals in living systems has only begun to be realized. No complete review of our present state of knowledge of liquid crystals in living systems is available. Stewart (1966, 1969) has given some insights into the role of liquid crystals *in vivo*. In a paper by Fergason and Brown (1968), an attempt is made to explain the role of liquid crystals in sensory systems, in cellular shape, and in the transmission of information.

CLASSIFICATION OF LIQUID CRYSTALS

There are two recognized classes of liquid crystals, namely, thermotropic and lyotropic. Thermotropic liquid crystals are those which are formed by heating the sample. Lyotropic liquid crystals are formed by mixing two or more components, one of which is generally highly polar (*e.g.*, water). Living systems fall in this latter class.

The molecules which form thermotropic liquid crystals possess certain features of common geometry, even though they are quite different stoichometrically. These molecular features may be summarized as follows.

The molecule is elongated and rectilinear. If the molecule has flat segments, *e.g.*, benzene rings, liquid crystallinity is generally enhanced. The ratio of molecular length to width is large.

The molecule is rigid along its long axis; double bonds are commonly found along this axis.

Simultaneous existence of strong dipoles and easily polarizable groups in the molecule seem important. The most pronounced liquid crystallinity is most likely to occur if the strong dipole is on the molecular axis.

Weak dipolar groups at the extremities of the molecule are of subordinate importance.

A more detailed description of molecular geometry in liquid crystallinity may be found in the writings of Gray (1962, 1969). Kast compiled a list of thermotropic liquid crystalline compounds (Kast, 1960). Even though this listing was compiled 10 years ago, it is the most complete listing available.

Thermotropic liquid crystals are subdivided into two

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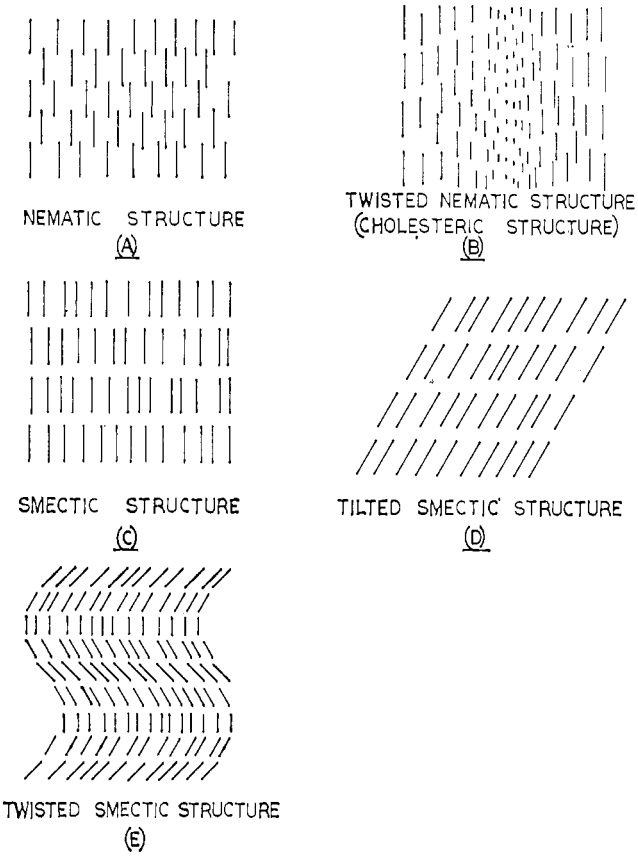

Figure 1. (A) Schematic diagram of molecular arrangement in uniformly oriented nematic liquid. Uniformly oriented structure has infinite-fold symmetry axis. (B) Schematic diagram of molecular arrangement in uniformly oriented cholesteric liquid. Infinite-fold screw axis. (C) Schematic diagram of smectic liquid. Infinite-fold symmetry axis. (D) Schematic diagram of molecular arrangement in tilted smectic structure. (E) Schematic diagram of molecular arrangement in twisted smectic structure

groups—nematic and smectic. A special kind of nematic liquid crystal is the twisted nematic or more commonly identified as the cholesteric liquid crystal.

A brief presentation of the structural characteristics of thermotropic liquid crystals seems in order. In the first place, there is a greater wealth of information about thermotropic liquid crystals than about lyotropic ones. Secondly, the well-known characteristics which we present for thermotropic liquid crystals are related to those of the lyotropic type.

SUBDIVISIONS OF THERMOTROPIC LIQUID CRYSTALS AND SOME OF THEIR PROPERTIES

Nematic Liquids. Nematic liquids differ structurally from isotropic liquids in the spontaneous orientation of the molecules along their long axes. Within the confines of a container and in the absence of external forces, the preferred direction of the long axes of the molecules is not constant, but generally varies continuously with position in the system. The optical axis in a nematic liquid coincides with the preferred direction of the long axes of the molecules. The nematic liquid is not optically active (bulk sample), but if it is placed between two glass plates and one is rotated slightly, the deformation of the structure by adhesion to the glass surface may result in an optically active system.

Nematic liquids, depending on the compounds, may exhibit different textures, *e.g.*, marbled textures or schlieren textures. The most common texture is the schlieren or threaded one. The threads result from a particularly strong dependence of the preferred direction on position in the vicinity of the axis of the thread; there may be no preferred direction in the axis of the thread itself.

Molecules in a nematic liquid are not all parallel to each other because of thermal motion in the system. The extent of parallelism is measured by the ordering parameter, S, which may be expressed as $S = {}^1/_2 \langle 3 \cos^2 \theta - 1 \rangle$ where θ is the angle between the long axis of a molecule and the axis of preferred orientation which coincides with the symmetry axis in a uniformly oriented nematic liquid crystal. If there is perfect molecular orientation $S = 1$, while for the isotropic liquid $S = 0$. The value of S is dependent on temperature and decreases with an increase in temperature throughout the nematic liquid crystalline range. For nematic liquids S lies somewhere between 0.3 and 0.8.

Smectic Liquids. Of the different types of liquid crystals, smectic liquids seem to be the most likely to be found in living matter. Their structural characteristics can accommodate many of the structural and metabolic properties of living matter. Molecules in a smectic liquid are arranged in layers which give them a stratified structure. Smectic liquids exhibit positive birefringence. At least five different textures of smectic liquids have been defined. They have been designated as smectic A, B, C, D, and E. The most extensive work on the identification and classification of smectic liquid crystals is that by Sackmann and Demus (1969). The reader may find a summary of their work in a recent paper by Brown *et al.* (1970). Two of these are represented schematically in Figure 1 (smectic A is sketch C and smectic C is sketch D). Smectic A is the most common and is represented schematically in Figure 1 (C). In this texture, the centers of gravity of the molecules are arranged in planes perpendicular to the preferred direction of the long axes of the molecules. Thus, the long axes of the molecules are randomly arranged; one may think of a smectic liquid as a two-dimensional isotropic liquid within layers.

There are experimental studies that give strong evidence for the existence of smectic structures in living systems. The stratified packing found in the smectic A and smectic C structures is adaptable to cell membrane structures. Such properties as ion transfer through cell membranes, transfer of organic molecules, and catalytic processes in cell functions can be accommodated in the stratified structures (lamellar packing in lyotropic systems).

Addition of water to the lamellar packing found in inanimate systems ordinarily results in molecular rearrangements showing hexagonal packing (Fig. 3b). Electron micrographs (Robertson, 1963; Napolitano *et al.*, 1967) of cell membranes give strong evidence that some cell membranes have a hexagonal packing similar to that found in inanimate systems composed of proteins, lipids and water. Rods with hexagonal packing (Fig. 3b) are adaptable to cell functions, as cited previously.

Both the lamellar and hexagonal structures are found in lyotropic liquid crystals. The molecular arrangements in both cases can be considered as paralleling the smectic structures found in thermotropic liquid crystals.

Twisted Nematic or Cholesteric Liquid Crystals. The twisted nematic (cholesteric) liquid has a number of interesting properties. The cholesteric structure exhibits dichromism (one of the polarized components of light is selectively reflected more strongly than the other). This property accounts for the iridescent color in the cholesteric liquid.

The colors are dependent on the temperature, the chemical material, and the angle of the incident radiation. Cholesteric liquids, because of their molecular architecture, have the capacity to rotate polarized light to a very great degree. This rotation is due to the bulk structure of the material and not to the asymmetry at the molecular level.

The cholesteric liquid has the parallel orientation of the molecules along their long axes, but superimposed on the parallel orientation is a spontaneous and continuous twist. There is no entropy difference between the classical nematic liquid and the cholesteric liquid (twisted nematic liquid). An optically active molecule is necessary to form a stable cholesteric liquid. A planar cholesteric structure in thin layers with a uniform twist will possess a large optical activity and will reflect cricularly polarized light selectively. The preferred direction of molecular orientation in a homogeneously oriented layer of a cholesteric liquid crystal is not constant over the whole volume, but the preferred molecular direction is constant within parallel planes. Moving along a line perpendicular to the planes, the preferred direction rotates uniformly. The pitch of this rotation through 2π is generally found to lie between 0.2 and 20 μ. The pitch is usually temperature dependent and the temperature dependence may be positive or negative. The twist in cholesteric liquids, which results from their unique molecular packing, can be observed in the optical characteristics of homogeneously ordered layers.

Schematic representation of nematic, cholesteric, and some smectic liquids are presented in Figure 1. For more details on the structure of liquid crystals, the reader is directed to general papers on the subject (Brown, 1969a,b; Brown and Shaw, 1957; Saupe, 1968; Gray, 1962). Theories of liquid crystalline structures have been proposed and the reader may find them explained in several sources (Saupe, 1968; Brown, 1969b).

LYOTROPIC LIQUID CRYSTALS

By combining two or more components, liquid crystalline systems in great numbers can be prepared. These systems are known in the inanimate and animate worlds. Our consideration of the structure and properties of lyotropic liquid crystals will use amphiphilic compounds with water as representative examples. Amphiphilic compounds are characterized by having in the same molecule two parts which show greatly different solubility properties. One part of the molecule is hydrophilic, which tends to be soluble in water and insoluble in hydrocarbons, while the other part is lipophilic, which has the reverse solubility characteristics. Depending on the relative contribution of each part of the molecule, amphiphilic compounds may range from essentially hydrophilic to predominantly lipophilic. The amphiphilic molecules most likely to form liquid crystals with water are those with strong hydrophilic and lipophilic groups which are rather equally matched. In the formation of lyotropic liquid crystals from amphiphiles and water, order arises as a consequence of selective interactions among two or more species. Typical hydrophilic groups are —OH, —$O(CH_2—CH_2—O)_nH$, —CO_2H, —CO_2Na, —SO_3K, —NMe_3Br, —$PO_4—CH_2—CH_2—NH_3^+$; and typical lipophilic groups are —C_nH_{2n+1} and —$O_2C—CH—CH_2CO_2C_nH_{2n+1}$.

A systematic classification of lyotropic liquid crystals has been developed by Luzzati and Skoulios and their coworkers (Luzzati and Reiss-Husson, 1966, 1967; Luzzati *et al.*, 1960). These classes are characterized by their optical properties and their structure (generally determined by X-ray). A schematic representation of three different types of molecular packings is presented in Figures 2 and 3. The lamellar packing in Figure 2a resembles the molecular packing in a smectic A thermotropic liquid crystal. The isotropic phase (cubic packing) in Figure 3a corresponds to the molecular packing proposed, but not yet proven, for a smectic D thermotropic liquid crystal.

The hexagonal packing represented in Figure 3b is common among polypeptide systems. The structure consists of a hexagonal array of parallel rods, with the solvent uniformly distributed among the rods. The packing in the two-dimensional lattice, as shown by the number of equatorial reflections, is different in different systems, with the number of those reflections as small as 3 and as large as 10. In some cases no equatorial reflections occur, suggesting some degree of lengthwise intermolecular interactions.

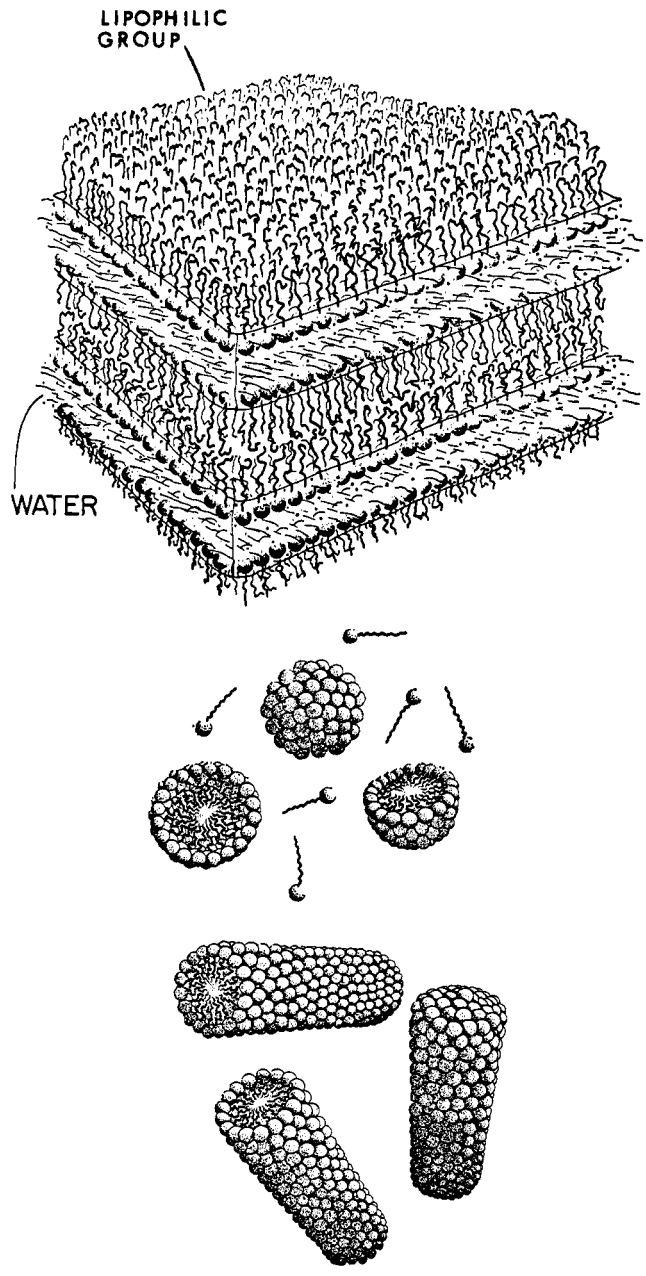

Figure 2. (Top) Schematic representation of the lamellar structure. (Bottom) Schematic representation of rod-like and spherical particles Rosevear (1968). Reproduced by permission of the *Journal of the Society of Cosmetic Chemists*

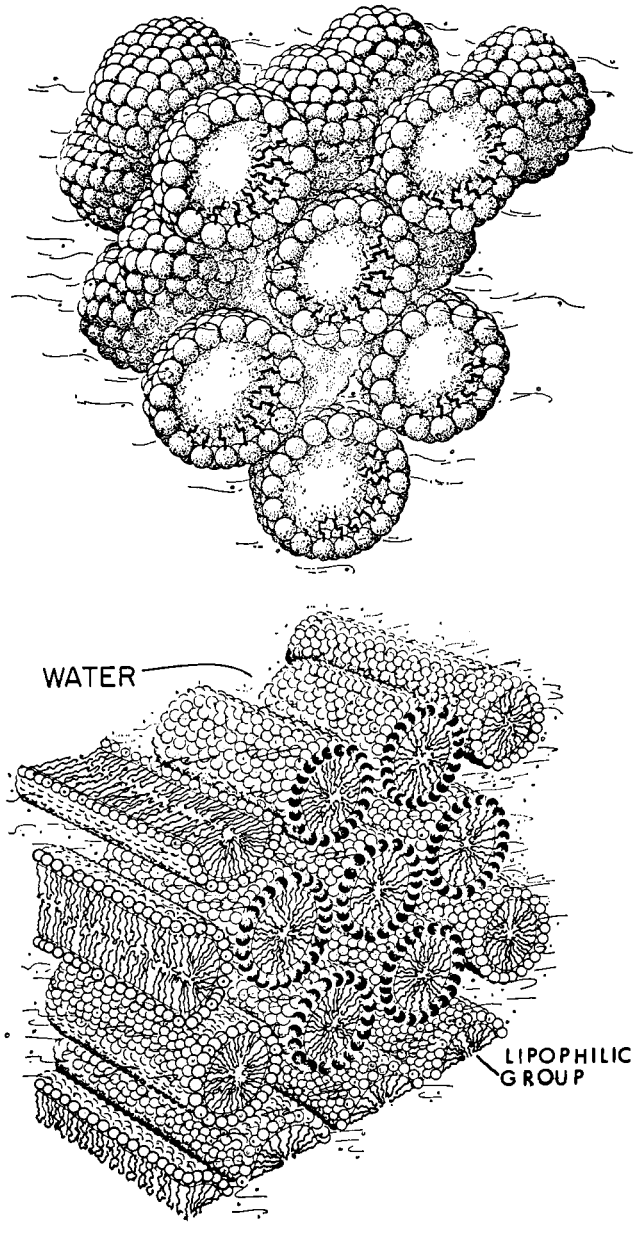

Figure 3. (Top) Schematic representation of the packing pattern of an isotropic phase. (Bottom) Schematic representation of the packing pattern of rod-like particles Rosevear (1968). Reproduced by permission of the *Journal of the Society of Cosmetic Chemists*

The hexagonal packing (Fig. 3b) can have six rods arranged on the corners of a hexagon which is a regular defect structure with $^1/_3$ of the structural units missing. If the seventh rod is placed inside the frame of the six rods in the hexagonal packing, a normal close-packed hexagonal structure satisfying the crystallographic requirements of three structural units per hexagonal cell is formed. The size of the hexagonal base of the unit cell and the X-ray intensities distribution indicate that more than one polypeptide molecule is associated with each two-dimensional unit cell. In one case, Saludjian and Luzzati (1967) concluded that X-ray data were consistent with the structure formed by a hexagonal array of bundles of three α helices. In this case, order in the system is represented by a parallel orientation. It is possible that the three helices in each aggregate are twisted around a common axis and take on a coiled conformation.

Another type of packing common in polypeptides is the tetragonal arrangement (ω form). The X-ray pattern of the arrangement contains several sharp reflections, the spacings of which can be indexed on a three-dimensional tetragonal cell; the length along the c axis is approximately 5.3 Å (Saludjian and Luzzati, 1967). The packing of the polypeptide is in a square lattice; the space group is $P4_1$ when the molecules are parallel and antiparallel if the space group is $P4_12_12$. This ω form was first observed by Bradbury *et al.* (1962) in certain fibers of PBLA, and by Fraser *et al.* (1962) in oriented films of poly-*S*-benzylthio-L-cysteine.

Information on the properties of the different lyotropic systems is cataloged in Table I. The systems which have molecular packings to give lamellar structures are represented with the symbol L with an appropriate subscript to distinguish one molecular arrangement from another. Those with the cubic packing will be identified with the letter C, and those with "particle structure" with the letter P. Appropriate subscripts are used in both structures to distinguish between molecular arrangements within a class.

There are two ways in which water-containing mesophases can form. The normal type has the organic portions of the molecules projected toward the center of the rod-like or spherical particles, while the hydrophilic portion is projected outward and surrounded by water. The reversed type has the opposite orientation of the molecules with the water inside the core of the rod. There are three excellent reviews (Lawrence, 1969; Winsor, 1968; Ekwall *et al.*, 1969) which contain details of the items mentioned in this paragraph.

Even though the arrangement of the molecular aggregates is quite well known, there is little reliable information of the arrangement of the molecules within the aggregates. We know that the hydrophilic groups on the amphiphile molecules are soluble in water and are thus fixed in the water-amphiphile interface. It seems reasonable, therefore, to assume that the organic portion of the molecules has an average orientation which may vary from phase to phase. Further considerations of lyotropic systems are discussed in a review by Brown *et al.* (1970).

Living systems are of the lyotropic class. We shall proceed to consider protein systems which have been shown to be liquid crystalline, and then we shall suggest a number of possibilities of proteins which might form liquid crystalline systems if the environment is proper.

SOME POLYPEPTIDE SYSTEMS AS LIQUID CRYSTALS

Introduction. The bonding forces between molecules in lyotropic systems are not yet clearly defined. Certainly, the bonding energies are of such a low value that one can eliminate the high-energy covalent bond. Hydrogen bonding, dispersion forces, dipole-dipole interactions, dipole-induced dipole interactions, and ion-dipole interactions represent the the kinds of intermolecular interactions to be expected in lyotropic liquid crystals.

While hydrogen bonding has not been seriously considered in the explanation of intermolecular interactions in thermotropic systems this bond cannot be excluded when one interprets the association between different molecules or segments of molecules in lyotropic systems. In case of biologic systems, the tertiary conformations are often governed by intramolecular hydrogen bonds. Intermolecular hydrogen bonding may give rise to structures like the pleated sheet of β-structures proposed by Pauling *et al.* (1951).

In thermotropic liquid crystals, the elongated shape of molecules which are flat or lathe-like is considered an important feature of their molecular geometry. This molecular shape does not seem necessary in lyotropic systems where

Table I. Different Types of Lyotropic Liquid Crystalline Phases[a]

Structural arrangement displaying Bragg spacing ratio $1:{}^1/_2:{}^1/_3$ with one-dimensional symmetry (one-dimensional periodicity)

Class	Description	Common Notation in Literature
L_1	Lamellar packing with coherent double layers of molecules and ions separated by water. Neat phase type	Neat phase
L_2	Lamellar packing with coherent single layers of molecules and ions separated by water. Single-layered lamellar type	
L_3	Lamellar packing with coherent double layers of molecules and ions separated by water. Mucous woven type	

Lyotropic liquid crystals with particle structure displaying Bragg spacing ratio $1:{}^1/_2:{}^1/_3:{}^1/_4$

Class	Description	Common Notation in Literature
P_1	Rod-like particles with organic core surrounded with water. Rods with predominantly quadratic cross-section in tetragonal arrangement. Normal two-dimensional tetragonal type	White phase
P_2	Rod-like particles with water core in organic environment. Rods with predominantly quadratic cross-section in tetragonal arrangement. Reversed two-dimensional tetragonal type	
P_3	Rod-like particles with organic core in aqueous environment. Rods with rectangular cross-section in an orthorhombic array. Normal two-dimensional rectangular type	Rectangular phase

Lyotropic liquid crystals displaying Bragg spacing ratio $1:{}^1/_3:{}^1/_4:{}^1/_7$ particle structure with molecules arranged in two-dimensional hexagonal symmetry

Class	Description	Common Notation in Literature
P_{H-1}	Rod-like particles with organic core in aqueous environment. Cylindrical to hexagonal cross-section in hexagonal array. Middle phase type; normal two-dimensional hexagonal type	(1) Middle phase (2) Hexagonal phase-I
P_{H-2}	Rod-like particles with aqueous core in organic environment. Cylindrical to hexagonal cross-section in hexagonal array. Reversed two-dimensional hexagonal type	Hexagonal phase-II
P_{H-3}	Rod-like particles with complex structure in aqueous environment. Complex two-dimensional hexagonal type	Complex hexagonal phase

Lyotropic liquid crystals displaying cubic symmetry

Isotropic lyotropic liquid crystals with spherical to dodecahedral particles arranged in face-centered cubic lattice

Class	Description	Common Notation in Literature
C_{f-1}	Particles with organic core in aqueous environment. Normal face-centered cubic type	Cubic phase, C_{I-1}
C_{f-2}	Particles with water core in organic environment. Reversed face-centered cubic type	Cubic phase, C_{I-2}
C_{f-3}	Particles with complex structure. Complex face-centered cubic type	

Isotropic lyotropic liquid crystals with spherical particles packed in body-centered cubic lattice

Class	Description
C_{b-1}	Particles with organic core in aqueous environment. Normal body-centered cubic type
C_{b-2}	Particles with complex structure. Complex body-centered cubic type

[a] Brown *et al.* (1970). Reproduced by permission of the Chemical Rubber Co., Cleveland, Ohio.

globular proteins like hemoglobin SS may exhibit liquid crystalline behavior under proper conditions.

It is also to be expected that there may be regions of liquid crystalline structure in macromolecules mixed with water and/or other solvents. Also, assemblage of several macromolecular units may be brought about by solvents, and in doing so generate liquid crystalline structures.

With the above background, one may now examine whether proteins do manifest any liquid crystalline associations. When one begins to consider polypeptides, the following ordered structures have been observed when appropriate environmental conditions are provided: the right handed α helix; the parallel and antiparallel pleated sheet β-structures; and the collagen-type fold. These ordered structures have been seen mostly in fibrous proteins. Among globular proteins, the α helix has been observed in myoglobin and there are strong grounds for inferring it in hemoglobin. The π helix may be possible. Luzzati *et al.* (1961) have suggested the existence of the 3_{10} helix due to relatively long spacings observed of poly-γ-benzyl-L-glutamates in certain solvents in dilute form.

Examples of Proteins Forming Liquid Crystalline Systems. We shall give some specific examples of proteins which form liquid crystalline systems. One of the classical studies of proteins as a liquid crystalline system was carried out by Robinson (1967), when he dissolved poly-γ-benzyl-L-glutamate in several different solvents. These systems show microscopically visible periodicities and a very high optical rotatory power comparable to that found in thermotropic cholesteric structures, the optical rotation and the periodicity being equal to half the pitch of the helix. The theoretical interpretation of these optical properties was developed by de Vries (1951). Other polypeptides also show this property. A number have been described by Saludjian and Luzzati (1967). The polypeptides which have been studied include: poly-γ-benzyl-L-glutamate (PBLG), poly-γ-methyl-L-glutamate (PMLG), poly-γ-ethyl-L-glutamate (PELG), poly-β-benzyl-L-aspartate (PBLA), poly-α-L-glutamic acid (PLGA), poly-α-sodium-L-glutamate (PLNaG) and, poly-L-lysine hydrochloride (PLL·HCl).

These polypeptides in solvents such as dimethylformamide (DMF), methylene chloride, water, hydrazine, and pyridine

Table II. Characteristics of the Various Forms of Polypeptides

Helix	Name	Rise per residue Å	Pitch Å	Instability kcal/mole per residue	Proposed by
2.2_7	α''-fold	2.75	6.05	0.5	Huggins (1943) Ambrose and Hanby (1949) Bragg *et al.* (1950)
3.0_{10}	...	2.00	6.00	1.0	Donohue (1953) Luzzati *et al.* (1961)
3.6_{13}	α-helix	1.50	5.40	0.0	Pauling *et al.* (1951)
4.3_{14}	...	1.20	5.16	2.4	Donohue (1953)
4.4_{16}	π-helix	1.15	5.06	0.5	Low and Grenville-Wells (1953)
5.1_7	γ-helix	0.98	5.00	2.0	Pauling *et al.* (1951) Kendrew (1963)

form structures which have the properties of the cholesteric structure (twisted nematic). These structures may be looked upon as having been derived from nematic liquid crystalline arrangements by imposing on them an axis of torsion at right angles to the long axis of the polypeptide molecule. Since the pitch of the helix is large compared to the distance between the molecules, the molecules in any small unit of volume will be nearly parallel.

The structure of the polypeptides in solution has been confirmed by a number of workers (Robinson, 1967) as follows.

X-ray diffraction shows that the molecules are essentially parallel.

The concentration of solute at which phase separation takes place is given by Flory's equation for suspended parallel rods.

In selected solvent mixtures, the structure untwists to give a nematic structure.

When the pitch is large compared to the wavelength of light, the experimental value of the optical rotatory power agrees with the de Vries model.

The properties predicted by the de Vries theory when the pitch is small have been found in some of these polypeptide solutions.

The reader is referred to Robinson (1967) for details.

Saludjian and Luzzati (1967) have made some interesting observations on the structures of polypeptides in different solvents. In brief, their conclusions may be summarized as follows. In most systems of polypeptides mentioned above, an extended concentration range is observed in the phase diagram over which variable amounts of solvent can be accommodated between the polypeptide molecules. In some systems, the conformation of the polypeptide molecules remains the same (α helical) over the range in spite of the difference in intermolecular organization found in the different phases (isotropic, cholesteric, hexagonal). In other systems the polypeptide conformation can be the α helix or the β form, depending on the concentration and the transition $\alpha \rightleftarrows \beta$ being reversible without an intermediate conformation. It is interesting to note that the concentration-dependent $\alpha \rightleftarrows \beta$ transition is observed only with polyelectrolytes.

The complex hexagonal phase with polypeptide molecules associated in bundles is stabilized by precise and specific solute–solvent and solute–solute interactions. This is confirmed by the fact that if the temperature is raised, the hexagonal structure collapses, giving way to the cholesteric phase. Further evidence on the specificity of the interactions is found in the equilibrium with the isotropic phase (PBLG–DMF) and with the cholesteric phase in another system (PBLG–pyridine).

The α helix and the β form are the most common conformations of polypeptides, but other geometric forms exist. The 4_{13} helix can be visualized as derived from the α helix by distortion produced by the lattice interactions. These somehow force the 3.6 screw axis of the α helix to become of order 4. The symmetry of the helix is utilized by the symmetry of the lattice. The intermolecular interactions enhance the stability of this conformation. Other conformations involving polypeptides claimed by Luzzati include the 3_{10} helix, and α'' ribbon and π helix.

Aqueous solutions of tobacco mosaic virus (Bernal and Fankuchen, 1937) have been shown to possess a mesomorphic state. The rod-shaped virus protein has been shown by X-ray to be arranged in a hexagonal close-packed array of parallel cylinders. As the concentration of the virus increases, the distance between adjacent molecules continuously decreases from the order of 500 Å to about 125 Å. When the molecules show the smectic structure, the layers are perhaps a thousand molecules thick (Bernal and Fankuchen, 1937).

Properties of Polypeptide Solutions Which are Characteristic of Liquid Crystals. The role of liquid crystals in protein chemistry is virtually unexplored. The protein chemists and the liquid crystal scientists have not joined forces in this potentially fertile field of research. In this section we shall describe the properties of a number of proteins which indicate that these polypeptides with a variety of solvents are potentially liquid crystalline systems. It should be made clear that the systems presented in this section have not necessarily been proven to be liquid crystalline, but afford research activities for those who are interested.

The stability of the various forms of polypeptides is suggested by data in Table II. The small energies mentioned are in the range of transitions of one liquid crystalline phase to another or to other kinds of phases.

The helix parameters are sensitive to the dihedral angles between the nitrogen–carbon (τ_{N-C}) and carbon–carbon (τ_{C-C}). Data correlating these parameters are given in Table III.

Table II documents the instability of various helical forms as influenced by bond lengths and angles. The factors which maintain the conformations of these helices are derived from the environment. Pauling *et al.* (1951) originally conceived the helices as being stabilized by a maximum number of linear hydrogen bonds and adherence to bond angles and lengths, as determined in small molecules containing the atoms in question. We now know that forces other than these play important roles in generating and stabilizing these helices

Table III. Relationship of Dihedral Angles and Helix Parameters

Structure	Description of conditions	Angle N-Cα-C′	Residues per turn	Axial rise per residue Å	τ_{N-C}	τ_{C-C}
α-Helix	Standard α-helix	110°	3.60	1.50	56¼°	48¼°
	From Trotter and Brown (1956)	110°	3.615	1.495	59½°	45½°
	Deuterated polybenzyl-L-aspartate left-handed helix	110°	3.61	1.504	55¾°	49°
	B-helix of myoglobin	110°	3.71	1.47	48¾°	58¾°
	If N-Cα-C′ is changed to 109°	109°	3.60	1.50	46°	57°
π-Helix	Helix by Low and Grenville-Wells (1953)	115°	4.4	1.15	57°	69½°
	If N-Cα-C′ is changed to 110°	110°	4.4	1.15	30½°	96½°

in solution. These include chain length of polypeptides, hydrophobic bonds, and the general solvophobic forces, side-chain hydrogen bonds, π-interaction of aromatic units, and the influence of ions. The terms hydrophobic bonds and solvophobic forces include the contributions of entropy effects, as visualized by Kauzmann (1954, 1959).

The preceding remarks suggest that polypeptide molecules exhibit weak intramolecular associations. These interactions may be solvent–solute and/or solute–solute. Specific conformations can occur by specific changes in conditions. For example, a ω helix conformation can be obtained from an α helix by a slight variation of conditions. Also, the $\alpha \rightleftarrows \beta$ transition is common. This transition may be initiated by water, pH, and change in concentration of the polypeptide. As in the case of thermotropic liquids, pressure and mechanical work such as rolling may induce transitions such as $\alpha \rightleftarrows \beta$, as found in the case of poly-DL-alanine (Elliott, 1952). Transformation of the helix to a random coil has been likened to melting (Joly, 1965) and is influenced by heat, pressure, mechanical work, and ionic strength.

Reagents, like urea and guanidine hydrochloride, which break hydrogen bonds cause denaturation and random coil formation. Detergents and surface active agents may cause denaturization by penetrating the hydrophobic interior of proteins, causing random coil formation. Sometimes polypeptides may have α helical forms in nonaqueous solvents and random coil forms in water. For example, poly-N^5 (ω hydroxyalkyl)-L-glutamates possess α helical forms in dimethylformamide, but show a partial helical form in water (Lupu-Lotan *et al.*, 1966), the degree of helicity increased by lowering the temperatures.

Optical properties of helical and other structures reflect the alignment of molecular segments in preferred directions in space, as shown by the following representative table (Table IV) of optical rotatary dispersions of L-polypeptides in various conformations. Solutions of proteins exhibit other properties characteristic of liquid crystals. These include circular dichromism, light scattering, and rheological properties.

Thus, we find that the helical forms of polypeptides are specific geometric forms, generated by solute–solvent and solute–solute interactions and other local influences. The forms are metastable and are transformed into other specific geometric forms with the involvement of relatively small amounts of energy. They possess optical and rheological properties characteristic of their asymmetric associations. Most of these properties are characteristic of both thermotropic and lyotropic liquid crystals.

Many naturally occurring proteins are known to exhibit certain helical packings. β forms have been observed in a variety of systems, such as silk, keratin, and porcupine quill. Lysozyme (Blake *et al.*, 1965) and various other proteins like β-lactoglobulin, γ-globulins, poly-L-lysine in water, and others are known to have β forms. Collagen with different solvents will form systems which may be in the forms of fibers or sheets (Schmitt, 1959). One could recite many other natural proteins which, in selected solvents, show liquid crystallinity or appear to possess the potential to show it. These are being examined in detail elsewhere in another paper. For example, we have taken arganine-rich histones and treated them with a defined amount of water to give a structure which we have shown by X-ray methods to exhibit a distorted crystalline packing. Additional water destroys the distorted crystalline structure, giving a new structure which is yet to be completely identified. Small amounts of impurities, in parts per million, will also destroy the packing patterns of the histone molecules in water.

Lyotropic liquid crystals involving proteins are found in inanimate and animate systems. Change in conformation may be involved in aging of enzymes, as well as in the activation of trysinogen and, indeed, allosteric effects in general. It seems reasonable that these conformations are in the form of liquid crystalline structures. If this be so there certainly is a widespread biological significance. Certain aspects of the structure of cell membranes where proteins are involved are sources of liquid crystalline systems (Ambrose, 1970; Fasman, 1967).

Table IV. Optical Rotatory Dispersion of L-Polypeptides

Conformation	Trough nm	Crossover nm	Peak nm	Reference
α-Helix	232–233	~224	198–199	Blout *et al.* (1962)
β-Form	299–23	~220	2.5	Iizuka and Yang (1966)
Random coil	238 (small)	...	228 (small)	Iizuka and Yang (1965)
	204–205	198	189	Blout *et al.* (1962)
Poly-L-proline II	210	203	196	Blout *et al.* (1963)

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