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A. C. Neville [a]
[a] Department of Zoology, University of Bristol, Woodland Road, Bristol, BS8 IUG
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Cholesteric Proteins

A. C. NEVILLE

Department of Zoology, University of Bristol, Woodland Road, Bristol BS8 1UG

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The properties of those proteins (mantis oothecal protein and sea cucumber collagen) which exist in biological systems as cholesteric liquid crystals are reviewed, together with some others (moth and fish eggshell proteins), which it is suspected pass through a cholesteric phase. Indices representing the overall degree of hydrophilicity have been calculated from the amino acid composition of mantis oothecal proteins. These indicate a very high net hydrophilicity which contrasts markedly with the net hydrophobicity of the isotropic oothecal proteins from cockroaches. The high hydrophilicity of mantis oothecins may cause the binding of water and so maintain the liquid crystalline state. Dehydration induced by quinone-tanning could subsequently lead to the solid crystals of the final product. The probable conformation of mantis oothecin is a coiled coil of two α-helices, which meets the requirements of a cholesteric liquid crystalline building block. Functional aspects of cholesteric proteins are discussed.

INTRODUCTION

Cholesteric liquid crystals and biological helicoids

The importance of liquid crystals in living organisms is becoming increasingly recognized.[1] Many biological structures are similar to those adopted by cholesteric liquid crystals. The cholesteric phase may be regarded as a twisted nematic state. This was convincingly demonstrated by Mauguin,[2] who produced cholesteric properties in a nematic mixture of azoxyanisole and azoxyphenetole by mechanical means. Observation through crossed polarizers showed that the long molecules were initially oriented in parallel. By twisting the nematic phase round and round between a microscope slide and coverslip, always in a constant sense of rotation, the preparation eventually developed interference colors, and reflected circularly polarized light of a sense appropriate to the direction of twist (features characteristic of the cholesteric state).

Helicoids are a type of structure commonly found in biological systems and which have similar construction to cholesterics. In a helicoid, each lamina has long components (either macromolecules or microfibrils) oriented in a parallel sheet. The grain direction in successive laminae is rotated in a screw sense

like the steps in a spiral staircase (Figure 1). Molecules which spontaneously self-assemble into cholesteric phases must possess chirality. Helical macromolecules, which are common in biology, are chiral and tend to pack naturally into helicoidal superstructures. When a helicoid is cut obliquely to its plane of lamination, the exposed face shows a pattern of arcs (Figure 1). Oblique sections, when viewed by transmission show similar patterns.

Any biological structure which is helicoidal is likely either to be cholesteric, or to have passed through a cholesteric phase during its development. Cholesteric structures usually lose their helicoidal ordering when they solidify. There are however some exceptions and these are of interest to developmental biologists. For instance, cholesteric glass can be made by rapidly cooling cholesteric liquid crystals of 2,4-dichlorocholesteryl benzoate.[3] Also, solid state samples of polybenzyl-L-glutamate have been prepared by using 3,3'-dimethylbiphenyl as a plasticizer.[4] Some preparations retained interference colors and reflected circularly polarized light (Samulski, pers. comm.). We have shown by electron microscopy[5] that it is possible to stabilize a cholesteric liquid crystal of preying mantis eggcase protein by fixation with glutaraldehyde. Self-ordering systems in biological materials are more economical in energy requirements than non-self-ordering structures which involve enzymatic control and the hydrolysis of energy-rich phosphate bonds in ATP. They are particularly appropriate for building skeletal parts, which lie mostly outside cells. Self-assembly can then be under remote control and regulated from the cells by extracellular variation of such simple factors as concentration of hydrogen or other ions.

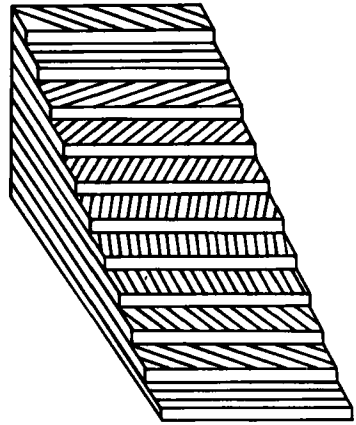

FIGURE 1 Diagram of a wedge of multiple ply laminate structure built of fibrous sheets. In each sheet the fibres lie in parallel. The direction of fibres in successive sheets changes through a small angle, always in a constant sense of rotation (here shown like a left-handed corkscrew), like the steps of a spiral staircase. This is called a ***helicoidal*** structure. A characteristic set of arcs is seen on the obliquely sectioned face. The sheets in the diagram rotate through 180°, generating one set of arcs: this represents the half pitch of the system.

That proteins dominate the properties of life is obvious. Yet our knowledge of those *proteins* which form cholesteric liquid crystals is in its infancy. Such information is scattered in the literature; the purpose here is to assemble and extend this knowledge, with particular emphasis upon the role of hydrophilic groups and the possible importance of water in mobility and subsequent stiffening.

DISCUSSION AND RESULTS

Examples of protein liquid crystals

It is well established that some synthetic polypeptides (e.g. polybenzyl glutamate, polymethyl glutamate, polyethyl glutamate and polybenzyl aspartate) can form cholesteric liquid crystals when dissolved in a variety of solvents.[6] It therefore came as no great surprise to us to discover that a naturally occurring protein does self-assemble into a cholesteric liquid crystal.[5] This first example was the protein from the enlarged left colleterial gland of female preying mantids (*Sphodromantis tenuidentata* and *Miomantis monarcha*), which is exuded to form the oothecal case around each batch of eggs. The extracted protein self-assembles *in vivo* and also *in vitro* on a glass surface. Electron micrographs of sections through the center of glutaraldehyde-fixed spherulites show a double spiral type of pattern (Figure 2), similar to that previously seen in spherical droplets of liquid crystals,[7] combined with the arced pattern interpreted as obliquely-sectioned helicoidal systems,[8,9] and independently observed in mantis proteins.[10] Three proteins have been isolated from freshly laid oothecae of the mantis *Tenodera sinensis.*[11] Oothecin I which represents 80% of the proteins present has a molecular weight of 43,000, while that of oothecin II is 60,000. The self-assembly of mantis oothecal protein has since been confirmed.[12] The

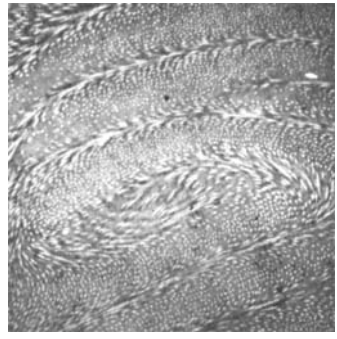

FIGURE 2 An electron micrograph of a section through the center of a cholesteric liquid crystalline spherulite of oothecal gland protein from a praying mantis (*Sphodromantis tenuidentata*), fixed *in situ* in the female egg case gland. The lamellae, between which run arced patterns of obliquely sectioned components, themselves appear to coil around in a double spiral typical of the center of a cholesteric liquid crystal spherulite. This is a typical picture of the center of a cholesteric spherical droplet. (×2,500).

proteins have been extracted, freeze dried in amyl acetate with solid carbon dioxide, and the water vapor sublimed off under vacuum. Water is required for self-assembly into the cholesteric phase. Since the material is still cholesteric in 0.2 M sodium sulphate, it seems that ionic interactions are not crucial. Furthermore, calcium ion concentration nor presence of EGTA affects the self-assembly process. The cholesteric phase changes to isotropic at pH 5.0.[12]

Frank[13] derived topological plots of fault patterns in cholesteric systems by consideration of free energy relations. The several types of disinclination which he predicted (χ consisting of paired cofocal parabolae and triple point; λ and τ faults in other planes; focal conics consisting of a paired ellipse and hyperbola) have all been seen in mantis oothecal protein self-assembled in 0.1 M acetic acid.[12]

The first example of collagen (the protein of bone and tendon) in a cholesteric liquid crystalline state has recently been reported).[14] This occurs in the Cuvierian organs of a sea cucumber (*Holothuria forskali:* Echinodermata). This protein is ejected as a sticky defensive secretion. The characteristic arced patterns are seen in electron micrographs of glutaraldehyde-fixed material. By contrast with the insect egg case proteins, sea cucumber collagen has polysaccharide glycosaminoglycans associated with it. These may be involved in determining the sense of rotation of the cholesteric collagen, because it is known that interference with their production by blocking L-glutamine synthesis with an antibiotic, 6-diazo-5-oxo-L-norleucine (DON) causes reversal of the collagen helicoid in the cornea of the eyes of 5 day old chicks.[15]

The above two examples are of proteins which are extractable in liquid crystalline form. Some other protein systems, while not being extractable as liquid crystals, are nevertheless suspected of assembling *via* a cholesteric phase.[16] These include silk moth eggshells;[17] salmon eggshell;[18] cod, plaice, and salmon trout eggshells;[19] and whelk periostracum.[18] Other systems which are believed to self-assemble *via* a cholesteric phase include insect cuticles[20] and plant cell walls.[21] In these cases it is not yet clear whether the polysaccharides (chitin and cellulose respectively) or the proteins are the chief architects.

The importance of water

Since the cholesteric phase of mantis oothecal proteins contains water, it is to be expected that changes in hydration will affect its structure. Hydration can indeed be altered by application of 200 atmospheres pressure. This increases the number of water molecules adsorbed on to the protein ionic groups, because adsorbed water occupies a lower volume than that in free solution,[12] and the phase becomes isotropic.

Whereas it is clear that synthetic polypeptides will form cholesteric liquid crystals in *non*-aqueous solvents, proteins in living systems operate in an aqueous environment. Heavily hydrated proteins have a high net hydrophilic character. Are cholesteric proteins more hydrophilic than non-cholesteric struc-

tural proteins? The hydration of a protein depends upon the balance between the hydrophilic and hydrophobic side chains of its amino acid residues. However, as pointed out by Andersen,[22] it is misleading simply to group the amino acids as hydrophilic or hydrophobic because of the wide quantitative range of these properties. The individual hydrophilicities have been calculated for each amino acid,[23,24] taking values derived from energy changes involved in transfer of residues from water to ethanol. These values, which are purely relative to an assumed value of zero for glycine, range from the most hydrophobic (tryptophan, + 3.4 kcal/residue) to the most hydrophilic (arginine and lysine, −3.0 kcal/residue). The dominant polar groups which have affinity for water by virtue of their hydrogen-bonding capabilities include —OH(1.0 kcal/mol), COO^- (4.5 kcal/mol) and —NHC $(NH_2)_2^+$ (6 kcal/mol).[23] Values of hydrophilicity (net negative totals) or hydrophobicity (net positive totals) have been calculated for various proteins from amino acid composition (Table I).

TABLE I

Calculated net hydrophilicity indices for cholesteric proteins (*), suspected cholesteric proteins (†) and some non-cholesteric structural proteins (**).

Net negative values indicate hydrophilicity; positive values hydrophobicity. The calculations are based upon amino acid contents taken from the references cited.

Species/protein	Net hydrophilic index
Tenodera sinensis (mantis)	
* Oothecin I[11]	−55.21
* Oothecin II[11]	−36.44
Sphodromantis centralis (mantis)	
* Total oothecal proteins[18]	−77.26
Salmo salar (salmon)	
† Eggshell chorion protein[18]	−11.98
Buccinum undatum (whelk)	
† Periostracum[18]	−33.98
Holothuria forskali (sea cucumber)	
* Cuvierian collagen[25]	− 4.97
Schistocerca gregaria (locust)	
** Resilin[26]	− 9.95
Homo sapiens (man)	
** Bone collagen[27]	+ 2.31
Silk moths (mean value)	
** Silk fibroins[18]	+16.24
Gallus domesticus (chicken)	
** Aorta wall elastin[28]	+67.13
Periplaneta americana (cockroach)	
** Isotropic eggcase proteins[29]	
A (Water insoluble)	+60.72
B (Water insoluble)	+78.72
C (Water insoluble)	+50.31
D (Water soluble)	+14.12
E (Water soluble)	+18.56
F (Water soluble)	+27.92

The cholesteric proteins, together with those proteins which are thought to self-assemble into solid helicoids via a cholesteric phase, do appear to be more hydrophilic than several non-cholesteric structural proteins. Perhaps this is associated with the mobility required to establish the cholesteric phase. The eggcase proteins of mantids and cockroaches (which are classified together in the same order, Dictyoptera) do appear to differ markedly in hydrophilicity, and perhaps this is related to other differences between them. Thus, prior to stiffening, mantis oothecins are birefringent and liquid crystalline, whereas those of cockroaches are in the form of isotropic globules. However, caution must be exercised in the interpretation of the small amount of available data.

The change from liquid crystalline to solid state

In their final form, mantis oothecal proteins are no longer liquid crystalline; they form a spongy solid state layer, which is no longer helicoidal, around the eggs. It is suggested here that dehydration may play an important part in this change of state, by exposing polar groups. These could then form hydrogen bonds between protein molecules. That hydrogen bonds are involved in self-assembly of the cholesteric phase can be deduced from the observation[12] that this is prevented by the presence of 8M urea, which, among other things, breaks hydrogen bonds.

The significance of controlled dehydration in the stiffening of insect cuticle has recently been re-emphasized.[30] Dried but untanned cuticle is just as stiff as cuticle which is both quinone-tanned and dried. Fraenkel and Rudall[31] had previously shown that dehydration is an active chemically controlled process, because cuticle still dehydrates even when submerged under water. Vincent and Hillerton[30] propose that there is a connection between quinone tanning and dehydration; they envisage displacement of water from hydrated groups by quinones, permitting hydrogen bond formation. Since it is known that quinones are also incorporated into freshly laid mantis egg cases,[32] the change from liquid crystalline to solid could equally well be mediated by quinone-controlled dehydration.

Functional aspects

When stabilized by hydrogen bonds and covalent cross-links, cholesteric systems form mechanically stable multiple ply laminates. These are ideal for construction of skeletal components and of the walls of spherical objects such as eggs. If such structures are squashed, the consequent rise in internal fluid pressure is resisted equally in all directions. Similarly the helicoidal arrangement of collagen fibers in the cornea of chicks is able to resist the pressure of fluid within the developing eye, so as to maintain a spherical surface.[15] Intraocular pressure is required for a developing cornea to assume normal curvature, without which vision would be defective.

The oothecal protein of mantids is extruded as a foam around the eggs. The foam is stabilized by its cholesteric structure.[12] It is known that helicoids can stabilize soap foams because their lamellar structure resists the thinning of the bubble walls.[33] Likewise, extracted oothecal protein can be shown experimentally to improve the stability of foams of sodium dodecyl sulphate by prolonging the life time of films before they pop.[12] Mantis oothecal foam is subsequently stiffened by hydrogen bonding and quinone-tanning.

What shape are the building blocks?

We can make some speculation about the possible shape of mantis oothecal proteins. Their chemical composition is consistent with a predominantly α-helical structure, with low quantities of helix-disrupting residues (proline, cysteine, glycine) and a high proportion of acidic amino acids (glutamic acid and aspartic acid). Mantis oothecal proteins suffer an irreversible denaturation at 55°C.[5] This might be attributed to the breakage of hydrogen bonds in the α-helices.

Although we do not know if or how the conformations of the proteins change from the liquid crystalline to the solid state, it is nevertheless relevant to summarize what is known about them. X-ray diffraction studies of mantis oothecal proteins were carried out either on the final fully crystallized product, or on bundles of fibres drawn from the liquid crystals and subsequently crystallized.[34,35] In both cases, the meridional X-ray reflections observed at 0.15 nm and 0.51 nm suggest a two stranded coiled coil of α-helices,[34] like that proposed by Crick for α-keratin.[36] The coiled coil is 1.8 nm thick and has a supercoil pitch of 16.5 nm.[35]

As with the muscle protein tropomyosin (which also consists of a double coil of α-helices),[37] a low proportion of non-polar (hydrophobic) amino acid residues would be predicted for mantis oothecins. Hydrophobic groups would pack together between the two chains, allowing more hydrogen bonds to form elsewhere, which is energetically favorable. A repeating sequence XXHXXXHXXHXXXH would be expected for coiled coils, where H is a hydrophobic amino acid residue, and X is any other.[38] This sequence has been confirmed for tropomyosin,[39] and represents 28.6% hydrophobic residues. My calculations of the total hydrophobic residues (tryptophan, phenylalanine, tyrosine, leucine, isoleucine, valine, proline, methionine, cysteine, and alanine) for mantis (*Tenodera sinensis*) oothecin I (30.1%) and oothecin II (32.5%) are compatible with this predicted sequence. It would now be of interest to determine the amino acid sequence of an oothecin.

It has been predicted that most polar residues will occupy the surface of a protein.[40] Proteins with a high polar content are thus likely to have a high axial ratio to accommodate these residues at the surface. We might then expect mantis oothecins to be long molecules, which fits with cholesteric require-

ments, and contrasts with the predominantly hydrophobic globular egg case proteins of cockroaches.

The estimated molecular weights of oothecin I (43,000) and oothecin II (60,000) are consistent with α-helical chain lengths of 65 nm and 85 nm respectively, with each coiled coil consisting of two α-helical chains. Coiled coils (or bundles of them) can well be visualized as spontaneously stacking in a helicoidal fashion, producing a cholesteric liquid crystalline state.

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