Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl16

Collagen Fibrils as Examples of Smectic A Biological Fibres

David W. L. Hukins [a] & John Woodhead-galloway [a b]
[a] Department of Medical Biophysics and Rheumatology, University of Manchester, Stopford Building, Manchester, M13 9PT
[b] MRC, 20 Park Crescent, London, WIN 4AL.
Version of record first published: 20 Apr 2011.

To cite this article: David W. L. Hukins & John Woodhead-galloway (1977): Collagen Fibrils as Examples of Smectic A Biological Fibres, Molecular Crystals and Liquid Crystals, 41:2, 33-39

To link to this article: http://dx.doi.org/10.1080/01406567708071950

Mol. Cryst. Liq. Cryst., Vol. 41 (Letters), pp. 33-39

Printed in the United States of America

COLLAGEN FIBRILS AS EXAMPLES OF SMECTIC A BIOLOGICAL FIBRES

DAVID W.L. HUKINS* and JOHN WOODHEAD-GALLOWAY†
Departments of Medical Biophysics* and Rheumatology†,
University of Manchester, Stopford Building, Manchester M13 9PT.

(Received 18 July 1977)

Collagen fibrils provide a biological example of smectic A liquid crystals. They demonstrate spiralling of their constituent chiral molecules, about the normal to their layers, when they undergo a transition to smectic C. Under tension, perpendicular to the planes of the layers, their molecules are tilted and some of them rearrange so as to describe a lattice whose unit cell has a square base. Novel features of the collagen fibril are that the layer thickness is dictated by the amino acid sequence of its collagen molecules, and not by their length, and that extra stability is conferred on the structure by covalent cross-links.

Collagen fibrils are a biological system which exemplifies the structural principles of smectic A (S_A) liquid crystals. Because of the selection pressures to which they are subjected during their evolution, biological structures often provide more interesting and sophisticated examples of physical-chemical principles than do synthetic systems. Collagen fibrils are unusual in that the thickness of the layers in their S_A structures is less than their molecular length and that extra tensile strength is conferred on the fibrils by covalent cross-links between the molecules. They also illustrate precession of the tilts of their chiral molecules in the transition to a smectic C (S_C) and, perhaps more interestingly, illustrate the formation of a square-based lattice when subjected to tensile stress.

Our purpose is to show that the collagen fibril furthers understanding of S_A liquid crystals. Collagen fibrils are found in the extracellular matrices of animal connective

† Present address: MRC, 20 Park Crescent, London WIN 4AL.

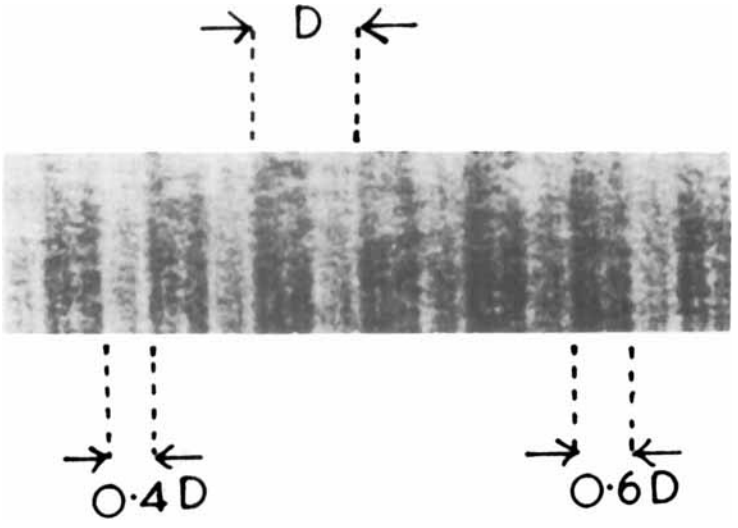

FIGURE 1 Electron micrograph of a "negatively stained"[1] collagen fibril from rat tail tendon. Sodium phosphotungstate (2% solution) was used as the stain. The thickness of a layer and two sub-layers are marked. In practice X-ray diffraction gives a better estimate of the thickness D than does electron microscopy[4].

tissues. They are roughly cylindrical and their length is several orders of magnitude greater than their diameter, which depends on the particular tissue but is typically of the order of 10^2nm. An electron micrograph of a "negatively stained"[1] fibril is shown in Figure 1. Collagen molecules can also be considered as cylinders with a diameter (1.2nm) which is much less than their length $(3.0 \times 10^2$ nm$)$[2]; more strictly they consist of three chains of L amino acids wound into coaxial helices and are, therefore, chiral[3]. Since collagen is a protein its molecules have distinguishable C and N terminal ends. We summarise the evidence which leads us to believe that collagen fibrils are S_A liquid crystals very briefly in the next paragraph. Further details and a consideration of the biological implications of our results will be published elsewhere.

The points of evidence on which we base our model for the structure of the collagen fibril are:

1. The meridian (parallel to the fibril axis) of low-angle X-ray diffraction patterns from oriented assemblies of collagen fibrils consists of orders of a 67 nm periodicity[4]. Thus the fibril consists of layers whose thickness is D=67nm.
2. Figure 1 shows that each layer consists of a sub-layer which can absorb stain (thickness 0.6D) and a sub-layer which excludes stain (thickness 0.4D)[5]. Since the molecular length is 4.4D this result is explained by each layer containing equal numbers of molecules with axial staggers of 0,1,2,3 and 4D as shown in Figure 2[5,6].
3. There is usually no long-range order in the side-to-side arrangement of molecules. The evidence for this assertion is provided by the equator (perpendicular to the meridian) of low-angle X-ray diffraction patterns from collagen fibrils from a variety of sources[7,8] (an exception occurs when fibrils are stretched - we return to this complication later).

Points (1) and (3) define an S_A liquid crystal. Point (2) shows that it has an unusual feature: layers consist of two sub-layers. The thicker sub-layer has a packing fraction which is 4/5 of that in the other sub-layer; therefore, roughly speaking, the former absorbs stain while the latter excludes it as in point (2). Figure 3 shows that this model is consistent with the equatorial intensity distribution of X-ray diffraction patterns from the collagen fibrils of fish fin-rays.

When collagen fibrils are dried their molecules sometimes spiral around the axis. This observation provides a further example of the behaviour of chiral molecules in the transition from S_A to S_C[9,10]. Experimental evidence for this transition has been obtained for collagen from a variety of sources using the techniques of electron microscopy[11,12] and X-ray diffraction[13,14]. We note that the transition occurs when the concentration of the long rod-shaped molecules changes i.e., they show typical lyotropic behaviour.

Under tensile stress collagen fibrils shown the expected S_A properties including the formation of a lattice with a square base, which have been predicted theoretically[15,16]. When rat tail tendons, which consist largely of oriented collagen fibrils, are stretched, Bragg reflections appear on the equator of their X-ray diffraction patterns. These reflections can be indexed on the basis of a lattice whose unit cell has a square base[17,18]. The layer thickness remains constant at D=67 nm and the molecules are tilted by about 4°.

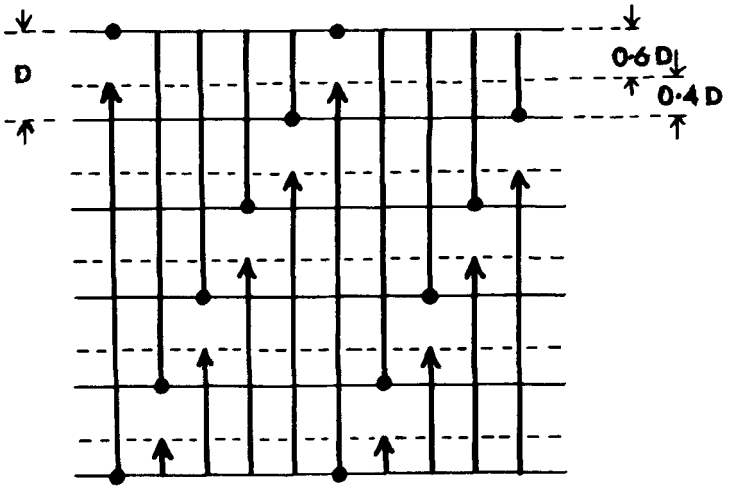

FIGURE 2 Model for the structure of a collagen fibril. Each layer contains equal numbers of molecules with axial staggers of 0,1,2,3 and 4D. The axially projected structure was solved using electron micrographs of negatively stained collagen[5] and confirmed using positively stained material[6]. Molecules are represented by arrows to show that they are all parallel (as opposed to anti-parallel). There is no long-range order in any plane perpendiculalar to the fibril axis.

from the fibril axis direction[19]. Recognition that the lattice arises from a distorted S_A liquid crystal suggests that two models proposed previously for the packing of molecules in the unit cell[17,18] are incorrect. We shall not pursue the matter here in case it should obscure our main argument.

It is worth noting that biological fibres might provide further examples of liquid crystals but, unfortunately, the methods generally used to study them could disguise their nature. X-Ray diffraction emphasises any ordered phase which may be present and electron microscopy tends to be subjective so that highly ordered structures receive greater attention. The suggestion that a biological structure might assemble itself like a liquid crystal is not new. Neville and his collaborators have demonstrated that arthropod cuticle might assemble via a cholesteric liquid crystal transition stage[20]. A similar suggestion has been made regarding the formation of chick corneal stroma[21].

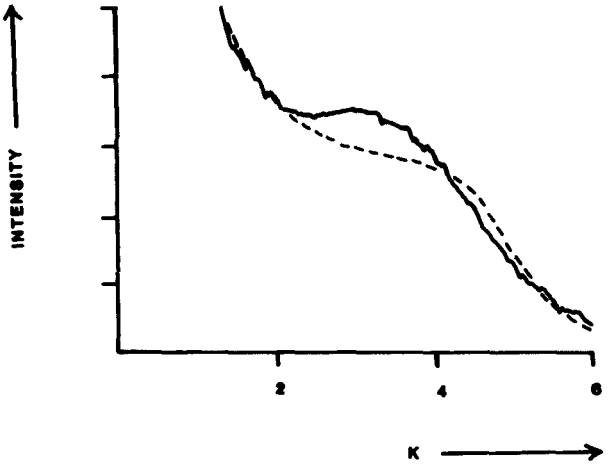

FIGURE 3 Comparison of observed low-angle X-ray diffraction equatorial intensity distribution with that predicted by the model of Figure 2. The intensity is on an arbitrary scale. K, the modulus of the scattering vector (measured in nm^{-1}) is defined by $K=(4\pi/\lambda)\sin(\phi/2)$ where λ is the X-ray wavelength (0.1542 nm) and ϕ is the angle through which the beam is scattered. The observed pattern (continuous line) was obtained from a fibril forming a fish fin-ray[13]. The calculated pattern (dashed line) was obtained by methods described elsewhere using a value of 0.47 for the two-dimensional packing fraction of the thinner sub-layer projected on to a plane perpendicular to the fibril axis; this packing fraction is consistent with density measurements[8].

We conclude by emphasing the novel features of the collagen structure. Although the discussion of the S_A to S_C transition and of the effect of tension provided information on the S_A state, the results were not altogether unexpected. Other aspects are more unusual and might be exploited in synthetic systems. The layer thickness in Figure 2 is dictated by the D-periodic sequence of charged and hydrophobic amino acid residues in the collagen molecule[22] and not by its length. Because the molecular length is not an integral multiple of the sequence periodicity each layer consists of two sub-layers. Collagen fibrils have to withstand tension in order to perform their

biological functions. The tensile strength of the fibril is enhanced by covalent cross-links between a molecule and a neighbour staggered by 4D with respect to it[23]. Hence the collagen fibril illustrates some expected and some unusual properties of S_A liquid crystals.

Acknowledgements: We thank G. Rogers and C.J. Eden for help with experiments and Professor P-G. de Gennes for information.

REFERENCES

1. S. Brenner and R.W. Horne, Biochim.Biophys.Acta, 34,103 (1959)
2. W.F. Harrington and P.H. van Hippel, Advan.Protein Chem, 16,1 (1961)
3. A. Rich and F.H.C. Crick, J.Mol.Biol. 3,483 (1961)
4. S.G. Tomlin and C.R. Worthington, Proc.Roy.Soc.,Ser.A, 235,189 (1965)
5. A.J. Hodge and J.A. Petruska in Aspects of Protein Structure G.N. Ramachandran, Ed., (Academic Press, New York, 1963) p.289.
6. J.A. Chapman and R.A. Hardcastle, Connective Tissue Res.2,137 (1974)
7. D.W.L. Hukins, Biochem.Biophys.Res.Commun. in press
8. J. Woodhead-Galloway, D.W.L. Hukins, D.P. Knight, P.A. Machin and J.B. Weiss, J.Mol.Biol. submitted
9. H. Sackmann and D. Demus, Mol.Cryst. 2,81 (1966)
10. W. Urbach and J.C. Billard, C.R.Hebd,Seanc.Acad.Sci., (Paris) 274, 1287 (1972)
11. C. Stolinski and A.C. Breathnach, J.Cell Sci. 23,325 (1977)
12. J.H. Lillie, D.K. MacCallum, L.J. Scalletta and J.C. Occhino, J. Ultrastructure Res. 58, 134 (1977)
13. D.W.L. Hukins, J. Woodhead-Galloway and D.P. Knight, Biochem.Biophys.Res.Commun. 73,1049 (1976)
14. J. Woodhead-Galloway and D.W.L. Hukins, J.Theor.Biol. submitted
15. J.M. Delrieu, J.Chem.Phys. 60, 1081 (1974)
16. P.-G. de Gennes, The Physics of Liquid Crystals (Clarendon Press, Oxford, 1974) p.284
17. A. Miller and D.A.D. Parry, J.Mol.Biol. 75, 441 (1973)
18. J. Woodhead-Galloway, D.W.L. Hukins and J.S. Wray, Biochem.Biophys.Res.Commun. 64, 1237 (1975)
19. R. Hosemann, R. Bonart and T. Nemetschek, Kolloid-Z.u.Z. Polymere, 252, 912 (1974)
20. A.C. Neville and B.M. Luke, J. Insect Physiol. 17, 519 (1971)

21. R.L. Trelstad and A.J. Coulombre, J.Cell Biol. 50, 840 (1971)
22. D.J.S. Hulmes, A. Miller, D.A.D. Parry, K.A. Piez and J. Woodhead-Galloway, J.Mol.Biol. 79, 137 (1973)
23. K. Kuhn, Essays in Biochemistry, 5, 59 (1969)