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Liquid Crystal Formation in Aqueous Solutions of a Polysaccharide Schizophyllan

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Concentrated aqueous solutions of a triple helical polysaccharide schizophyllan with molecular weight 4×10^5 were investigated by polarizing microscopy and laser light diffraction at 30°C. The solutions were birefringent above a polymer weight fraction w of about 0.098, indicating the formation of a liquid crystalline phase. This liquid crystalline phase resembled in many respects cholesteric mesophases reported for polypeptide liquid crystals, and was found to be also cholesteric. It was characterized by a cholesteric pitch of the order of several μm varying approximately in proportion to $w^{-1.9}$. The system studied was completely liquid crystalline above $w = 0.127$ but biphasic in the range of w between 0.098 and 0.127, and underwent an isotropic-liquid crystalline phase transition when the temperature was changed.

INTRODUCTION

Recently Norisuye *et al.*[1] concluded from viscosity and sedimentation equilibrium measurements that schizophyllan, an extracellular β-1,3-D-glucan produced by *schizophyllum commune,* exists in dilute aqueous solution as a rodlike triple helix. Subsequently they investigated dimensional and hydrodynamic properties of the triple helix in water and 0.01 N aqueous sodium hydroxide and showed that the triple helix is approximated by a wormlike chain of Kratky and Porod with a persistence length of about 180 nm.[2,3] For this remarkable rigidity comparable to that of collagen,[4] which is much more rigid than other stiff chain molecules, it can be expected that an aqueous solution of schizophyllan forms a liquid crystalline phase if the polymer concentration is increased to a certain value. Schizophyllan in water is optically active, and hence its triple helix should have a definite sense, although it is not established as yet. Thus it is expected that the liquid crystalline phase, if formed, may be cholesteric.

The present study was motivated by this expectation, and indeed, it was found from polarizing microscopy and laser light diffraction that this is the case with our aqueous solutions of schizophyllan. Experimental observations leading to this finding are described below.

EXPERIMENTAL

Two purified samples of schizophyllan provided by Taito Co. were used. They had been prepared by sonication of native schizophyllan followed by fractional precipitation.[1-3] Table I gives their molecular characteristics; the viscosity-average molecular weights M_v were calculated from intrinsic viscosities in water at 25°C using the viscosity-molecular weight relationship established by Yanaki *et al.*[2] Table I also lists the axial ratio of each sample estimated from the helix pitch per β-1,3-D-glucose unit 0.3 nm and the diameter of the helix 2.6 nm.[2] We note that the schizophyllan triple helix with a molecular weight of 4×10^5 is almost perfectly rigid and straight.

A required amount of a schizophyllan sample was dried in vacuo for two days and mixed with water in a stoppered flask of 3 ml capacity. The mixture became transparent within two days and appeared very viscous. Mixing was effected by rotating the flask and eventually a uniform transparent solution was obtained. Polymer weight fractions were determined gravimetrically.

Drum-shaped glass cells (diameter = 10 mm, thickness = 1 mm) with a thin inlet tube were used for both microscopic observation and laser light diffraction. A cell was filled with an appropriate amount of the solution, sealed off at the inlet tube, and stored at 30°C until use.

A Union Mec-3 microscope was used for microscopic observations. This microscope has a wide working distance between the objective and condensing lenses, thus permitting a thermostatic cell holder to be inserted there.

A He-Ne laser, a thermostatic cell holder, and a screen were arranged in this order on an optical bench. A glass cell was put into the cell holder which was fixed on the stage of a traveling microscope. Thus the cell was movable along the direction of laser beam and its position could be determined as accurately

TABLE I

Molecular characterization of the schizophyllan samples used

Sample code	$[\eta]$ / 100 cm^3 g^{-1}	M_v / g mol^{-1}	Axial ratio
D-4	4.0	390000	68
D-40	4.6	410000	72

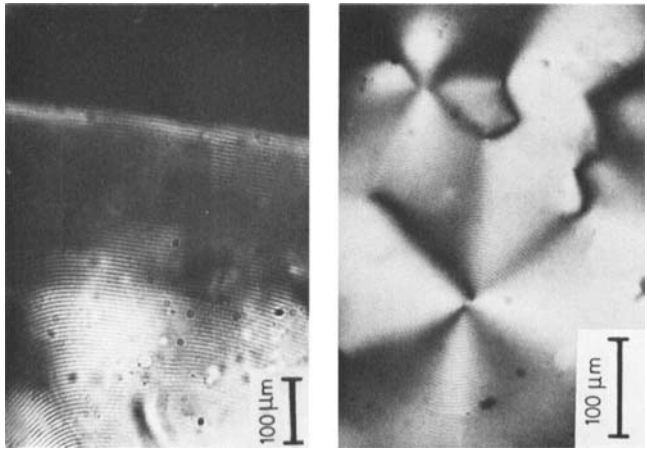
as ±0.002 cm. Diffraction patterns projected on the screen were measured directly by another traveling microscope or read from their photographs. Thermostated water was circulated through the cell holder to maintain the temperature of the solution constant within ±0.1°C in a range between 5 and 80°C.

Optical rotatory dispersion measurements were made on a JASCO ORD/UV-5 recording spectropolarimeter with quartz cells of 1 mm thickness.

EXPERIMENTAL RESULTS

Microscopic observation

Aqueous solutions of schizophyllan sealed in drum-shaped cells were examined under crossed polars. At low concentrations, the solutions appeared dark, indicating that they were isotropic. Above a certain concentration, however, they showed complicated patterns, being bright and dark and of various colors. About two hr after the solution had been introduced into the cell, alternate bright and dark lines began visible in various places in the cell. After the cell had been kept at 30°C for several weeks, these lines spread over the entire area of the cell. Figure 1 shows typical photographs of birefringent solutions. In panel (a) of Figure 1, fine parallel lines near the cell wall run parallel to it, while those far apart from it run in different directions. If these lines are considered to represent some layer structure, as in the case with polypeptide liquid crystals,[5] such layers must be arranged parallel to the cell wall in the former



(a) (b)

FIGURE 1 Microscopic photographs of aqueous solutions of schizophyllan between crossed polars. (a) Sample D-40, $w = 0.127$; (b) Sample D-4, $w = 0.183$. Unless specified all the photographs are those taken at room temperature around 25°C.

area. On the other hand, in the central area, parallel lines run in various directions, forming in some cases focal conic patterns as shown in panel (b) of Figure 1. The microscopic pattern looked different if the microscope was focused at different depths of the solution.

Similar observations were made at a number of areas and on different solutions. These observations are summarized as follows: (1) There are places where parallel lines are seen over a wide area, (2) the parallel lines curve up and down and disappear at some place when focused at a fixed depth, and (3) patterns with characteristic disclinations are observed. Figure 2 shows selected photographs which contain patterns resembling closely λ^+ or λ^- disclination observed in cholesterics.[6,7]

Soon after the parallel lines began visible, their spacings appeared to change with one place to another and with time, too. But after several weeks, they acquired equilibrium values, which were practically constant over the entire area except for peripheral ones. The spacing was 3.70 ± 0.05 μm for the D-4 solution with polymer weight fraction $w = 0.183$ [panel (b) of Figure 1].

At a relatively low concentration the birefringent phase first appears as spherulites; a typical photograph of such spherulites is shown in Figure 3, where the directions of the polars are indicated by arrows. A Maltase cross is seen in the directions of the polars. On a birefringent solution introduced into a thin capillary tube were observed parallel lines in the direction of capillary axis. Measurement using a λ plate revealed that the higher refractive index is along these lines. Assuming that the higher refractive index of the schizophyllan triple helix is along its helix axis, we can conclude that the helix axis is tangential to the parallel lines, i.e., it is in each layer.

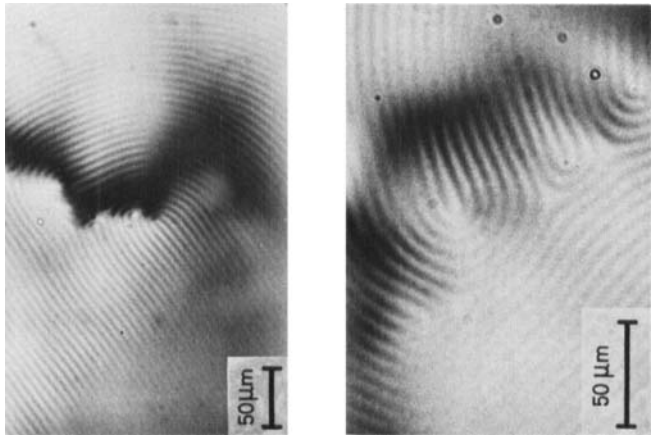


FIGURE 2 Microscopic textures in an aqueous solution of D-40 with $w = 0.127$.

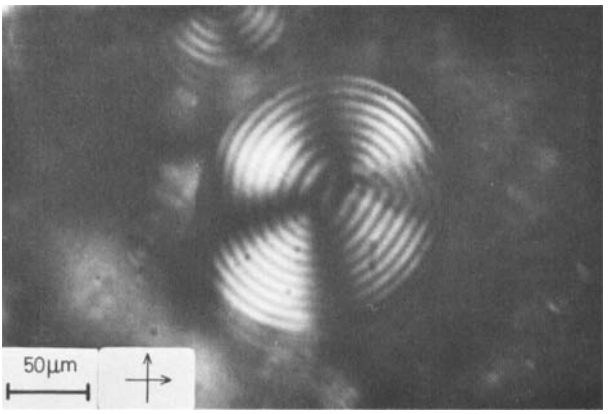


FIGURE 3 Large spherulite between crossed polars. Sample D-40, $w = 0.101$.

Laser Light Diffraction

A schizophyllan solution of relatively low concentration placed between crossed polars did not transmit laser light. Thus the solution was isotropic. However, a sufficiently concentrated solution depolarized laser light and transmitted it, which indicated that the solution was birefringent. When the solution was illuminated without polars, a diffraction ring was projected on the screen; one of typical examples is shown in Figure 4. These observations are quite similar to those reported for polypeptide liquid crystals,[5] and the diffraction ring must be associated with the multilayer structure disclosed above by microscopic observation. Therefore the spacing S between layers is related to the diffraction angle 2θ in the solution by[5]

$$2S \sin \theta = \lambda_0/n \tag{1}$$

where λ_0 is the wavelength of light in vacuo and n is the refractive index of the solution. As shown in Appendix, S is related to ϕ the angle between the direction of incident light and that of diffracted light in air by

$$S = \lambda_0 \cos \theta / \sin \phi \tag{2}$$

with

$$\cos \theta = \cos [(\tfrac{1}{2}) \sin^{-1} (n^{-1} \sin \phi)] \tag{3}$$

The angle ϕ can be determined from the distance between the screen and the outer cell wall x and the radius of the ring y using the relation

$$\tan \phi = (y_2 - y_1)/(x_2 - x_1) \tag{4}$$

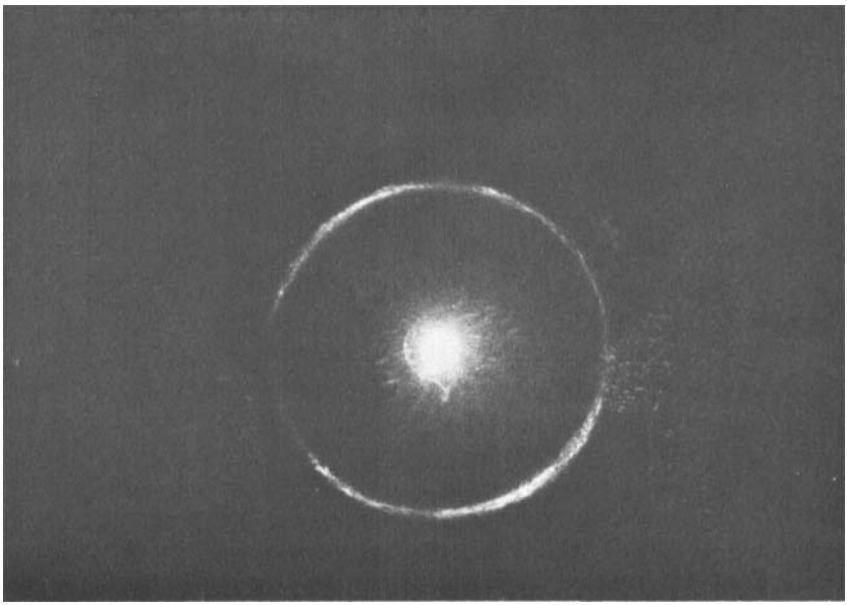


FIGURE 4 Diffraction pattern obtained for an aqueous solution of D-4 with $w = 0.183$. $S = 3.7$ μm.

where y_1 and y_2 are the y values for two different positions of the cell, x_1 and x_2, respectively. The correction factor $\cos\theta$ given by Eq. (3) is not much different from unity and not sensitive to n. In actual application to aqueous schizophyllan solutions, n was assumed to be 1.35.

As will be shown below, the values of S obtained in this way using Eq. (2) agree well with those from microscopic photographs mentioned above; they are accurate to $\pm 2\%$.

Robinson[5] reported for poly(γ-benzyl L-glutamate) in methylene chloride a diffraction pattern where the intensity of the rings fall off relatively gradually with successive orders and even eight orders are visible, indicating the presence of very regular structure. Similar observations are reported for other poly peptide-solvent systems.[8] These results are at variance with ours in which the second order diffraction was seldom detected. The reason for this discrepancy is not clear to us.

Spacing S as a Function of Polymer Concentration

Table II gives values of S at 30°C as a function of polymer weight fraction w. It can be seen that the S values obtained by polarizing microscope and diffraction are in excellent agreement. Figure 5 shows a double-logarithmic plot of S

TABLE II

Spacing S for a schizophyllan sample D-40 in water at 30°C

w	$S/10^{-4}$ cm microscope	diffraction
0.101	$7.1_1 \pm 0.7$	—
0.112	7.0_0	6.8_7
0.127	6.8_3	6.6_6
0.139	5.2_6	5.1_6
0.165	3.7_4	3.6_4
0.215	2.2_5	2.2_1
0.274	1.3_4	1.4_1
0.388	—	0.79

vs. w, where unfilled circles represent the data in the birefringent phase and half-filled circles represent those in the biphasic region; the straight line has a slope—1.9. Quite similar concentration dependence of S was first reported by Robinson *et al.*[9] for poly(γ-benzyl L-glutamate) and poly(γ-methyl L-glutamate), but polypeptide-solvent systems exhibiting similar or different concentration dependence are also reported.[10,11]

DISCUSSION

Since schizophyllan is optically active and its triple helix should have a definite sense, it is quite natural to expect that an aqueous solution of schizophyllan

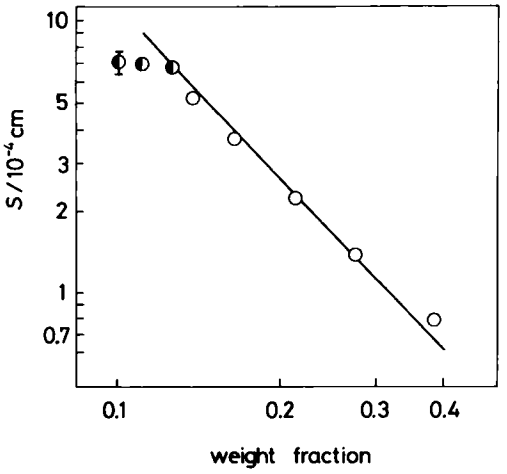


FIGURE 5 Concentration dependence of S for D-40 in water at 30°C. Unfilled circles, data in the liquid crystalline phase; half-filled circles, data in the two-phases region; the straight line, drawn to have a slope −1.9.

should form a cholesteric mesophase above some critical concentration. This expectation is substantiated by the following observations.

(1) Under crossed polars colored bright and dark patterns with alternate bright and dark lines are observed; the spacing between the lines is constant throughout the solution. The patterns exhibit some disclinations resembling closely those characteristic of cholesteric mesophases.

(2) When illuminated by laser light without polars, a birefringent solution gives diffraction rings, which are associated exactly with the spacing S observed by microscope. The dependence of S on polymer concentration for our system is essentially the same as those found by Robinson *et al.*[9] in polypeptide liquid crystals, which are of course cholesteric.

(3) As shown below, the birefringent phase is supposed to have very large optical rotation, which is another characteristic feature of cholesterics.[5,6,12,13]

Some polysaccharides and their derivatives are also known to form cholesteric mesophases,[14,15] although at concentrations higher than those with schizophyllan. Since these polysaccharides are considered to be much more flexible than schizophyllan and cannot be regarded as rigid molecules in themselves, the molecular mechanisms of mesophase formation operating in these two classes of polysaccharides may be different from each other.

Equilibrium between Isotropic and Liquid Crystalline Phases

Rough estimate on the basis of microscopic observation shows that aqueous solutions of schizophyllan are isotropic below $w = 0.0975$, biphasic between 0.0975 and 0.127, and liquid crystalline above 0.127. Solutions in the biphasic region look considerably turbid and exhibit microscopic patterns containing alternate bright and dark parallel lines and many circles of different sizes. The values of S determined from such patterns are also included in Figure 5 (half-filled circles). It should be noted that S stays almost constant in the biphasic region. With the assumption that S is a unique function of polymer concentration in the liquid crystalline phase, this fact is taken to mean that our system is separated into two phases of fixed concentrations, the volume ratio between the two varying with total polymer concentration.

When prepared and examined at 30°C, a solution of D-40 with $w = 0.101$ was biphasic and characterized by a specific S value 7.0 ± 0.5 μm. However, standing at this temperature for several days, it separated into two layers, the upper one being much less birefringent than the lower one. Figure 6 shows a microscopic photograph of the boundary between the two layers. The lower layer is in large part covered with systems of parallel lines; the lines form spherulites in some areas but they run through circular patterns in other areas. The circular patterns may be spherical regions of the isotropic phase trapped in the liquid crystalline phase. On the other hand, in the upper layer are seen many spherulites floating in a sea of the isotropic phase. It should be noted

FIGURE 6 Two-phase solution of D-40 with $w = 0.101$ at 30°C.

that the spacing S is substantially the same in both layers. This supports the argument presented above that both layers consist of the two phases, i.e., isotropic and liquid crystalline, of the concentrations just encompassing the biphasic region.

It was found that a solution of D-40 with $w = 0.0975$, which was isotropic at 40°C, turned biphasic when cooled down to 2°C. Its liquid crystalline phase was characterized by an S value nearly equal to those in the biphasic region at 30°C. On the other hand, a biphasic solution with $w = 0.112$ was heated to become isotropic at temperatures above 80°C but became biphasic again at 30°C. Standing at that temperature for two days, it separated into two layers just as in the case of $w = 0.101$. Thus we conclude that the system schizophyllan + water undergoes an isotropic-liquid crystalline phase transition with temperature as well as with concentration. Further discussion of phase relationship is deferred until quantitative data for phase volumes and compositions become available.

One of the most striking features of a cholesteric mesophase is its very large optical rotation.[5,6,12,13] Although we have not yet succeeded in directly measuring the optical rotation of the liquid crystalline phase, we have evidence which strongly suggests that this is also true with our schizophyllan solutions. When an isotropic D-40 solution with $w = 0.0955$ was cooled, it got phase-separated around 4.5°C. In a temperature range between 7.5 and 5.0°C, the optical rotation of the solution was found to increase by a factor of more than 20 with decreasing temperature and appeared to diverge at the phase separation temperature. This suggests that the liquid crystalline phase may have a very large optical rotatory power. A similar increase in optical rotation near the phase transition point has been reported recently by Patel and Dupré[16] for solutions of poly(γ-benzyl glutamate)s. This phenomenon with schizophyllan will be discussed in greater detail in a forthcoming paper of this series.[17]

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Appendix

We assume that a cholesteric solution consists of a system of diffraction gratings, which have a constant spacing S but are randomly oriented. Light traveling through the solution is diffracted when the Bragg condition Eq. (1) is satisfied.[5] Since both the incident and diffracted beams are refracted at interfaces between layers with different refractive indices, the direction of the diffracted beam in the solution is not always the same as that in air. Thus we need a correction for this refraction effect to deduce correct θ from experimentally obtainable data. Stein and Keane[18] developed a method for correcting a similar refraction effect in light scattering from thin films. Their method is applied to our case by noticing that our system is not a single layer like a film but consists of three layers, i.e., two glass walls and a cholesteric solution.

Correction for Refraction

Suppose that the incident beam is propagated through a glass cell with its surfaces facing perpendicularly to the propagation direction. The cell has two

parallel glass walls with thickness ϵ and refractive index n'. The incident beam is diffracted at point M in the solution into the direction which makes an angle 2θ with its original direction. It is then refracted at the solution-glass and glass-air interfaces to reach point R on the screen. The screen is placed perpendicularly to the direction of the incident beam at point P; the distance between P and the outer cell wall is denoted by x. Letting the incidence and refraction angles at the glass-air interface be ψ and ϕ respectively, we find

$$\sin 2\theta = \frac{1}{n} \sin \phi \tag{A.1}$$

Substitution of this equation into Eq. (1) gives Eq. (2). The angle ϕ is expressed in terms of x and the distance y between points R and P (i.e., the radius of the diffraction ring) as

$$\tan \phi = (1/x)\,(y - \delta) \tag{A.2}$$

with

$$\delta = d \tan 2\theta + \epsilon \tan \psi \tag{A.3}$$

where d is the distance between point M and inner cell wall at the exit end. Thus ϕ can be evaluated if y, δ, and x are determined, though accurate determination of these values is not necessarily easy.

If the cell is moved along the direction of the incident beam to obtain the values of x and y for two different cell positions which are denoted by x_i and y_i (i = 1,2), $\tan \phi$ for either of these cell positions is given by Eq. (A.2) with x and y replaced by x_i and y_i, respectively. Eliminating δ from the resulting two equations with the assumption that d is the same for both (see below for this assumption), we get Eq. (4). This equation may be preferred to Eq. (A.2), because the distances $y_2 - y_1$ and $x_2 - x_1$ may be determined more accurately than the absolute values of y, x, and δ.

Uncertainty due to the Finite Cell Thickness

Since the cell has a finite thickness d_0, the position R varies depending on where in the solution the incident beam is diffracted. This gives rise to a finite breadth for the diffraction ring, making the estimated y value inaccurate. Let y_e and y_0 be the y values for the diffracted beams coming from the two extreme positions, i.e., the entrance and exit of the cell, respectively. Then we have the equalities:

$$\tan \phi = (y_e - d_0 \tan 2\theta - \epsilon \tan \psi)/x = (y_0 - \epsilon \tan \psi)/x \tag{A.4}$$

which give

$$y_e - y_0 = d_0 \tan 2\theta \tag{A.5}$$

If y_e is used instead of the "correct" value y_0 in the latter of Eq. (A.4), an apparent ϕ value, ϕ', is obtained, which is related to ϕ by

$$\tan \phi' = [1 + (d_0/x) \tan 2\theta/\tan \phi] \tan \phi \tag{A.6}$$

The correction term $d_0 \tan 2\theta/(x \tan \phi)$ increases as either x or ϕ or both decrease; note that $\tan 2\theta/\tan \phi$ increases from zero to $1/n$ as ϕ decreases from $\pi/2$ to zero. However, even for an unfavorable set of parameters, i.e., $x = 50$ mm, $\phi \sim 0$, $d_0 = 1$ mm, and $n = 1.4$, this correction term is only 0.014, and hence $\tan \phi'$ can be equated to the required $\tan \phi$ within an error of less than 1.5%.

Oblique Incidence

Stein and Keane[18] derived an equation describing the refraction effect in light scattering from thin films. It is shown that their equation can be used in our case as well, although our system is not a single layer but a solution sandwiched between two glass plates. For an oblique incidence angle α, it reads in our notation

$$\sin (\phi + \alpha) = n \sin \left[2\theta + \sin^{-1} \left(\frac{1}{n} \sin \alpha \right) \right] \tag{A.7}$$

where 2θ and ϕ are measured anticlockwise from the direction of the undiffracted beam. This equation reduces to Eq. (A.1) for normal incidence, i.e., $\alpha = 0$.

According to Eq. (A.7), ϕ is an increasing function of α for a given value of θ, which implies that the apparent S value estimated by using Eq. (A.1) should decrease with increasing α. In a usual case with α as small as 5°C, the estimated S is substantially the same as the correct one. It should be remarked that the finite thickness of the cell walls has no consequence in either of the final expressions: Eqs. (2), (4), and (A.7).