

# Long-range order of nucleic acids in aqueous solutions

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(Received 25 October 1979)

*The structural order of short nucleic acid fragments (having a mean length of 170 nm) in aqueous solution in the presence of high magnetic fields (up to 18.5 tesla) has been investigated by small-angle neutron scattering, light diffraction and by precision measurements of the magnetic birefringence. Our data give clear evidence that, above a critical concentration, the semi-rigid electrically charged fragments arrange themselves into a periodic lattice having an interparticle spacing of ~6 nm. Neighbouring rods show a nearly parallel orientation, but a slight twist seems to exist, leading to a well defined pitch of the order of 1000 nm, which gives rise to a strong diffraction of visible light. The unexpectedly low saturation of the birefringence in the high magnetic field, however, indicates that the order is not of the simple cholesteric type. The forces which are responsible for inducing the twist across the large interparticle distance are mainly anisotropic Van der Waals forces.*

## Introduction

Macromolecular aqueous solutions and colloidal suspensions of large asymmetric biological particles may undergo a transition from the isotropic to various anisotropic phases when increasing the concentration beyond a well defined critical value. Examples range from tobacco mosaic virus<sup>1,2</sup>, bacteria<sup>3</sup>, myosin<sup>4</sup> and collagen<sup>5</sup> to flexible polyelectrolytes<sup>6,7</sup>, all forming *nematic* or *smectic* phases. Other helical particles, such as  $\alpha$ -helical polypeptides<sup>8-13</sup>, bacteriophages<sup>14</sup> and double helical nucleic acids<sup>15-19</sup> show, in addition, optically active, sometimes *cholesteric*, phases. Very recently, Jizuka has described a cholesteric phase of double-helical polynucleotides of high molecular weight in aqueous solutions<sup>17-19</sup>, which he studied by X-rays, optical diffraction and circular dichroism, including an investigation on the orientation of these long-chain molecules in magnetic fields. As pointed out before<sup>20,21</sup>, these ordered macromolecular systems are also of considerable biological interest.

From these results it seems clear that the interparticle distance is very large and that there exists a small but definite twist angle between neighbouring rods. But the macroscopic order is not well understood and it is unclear which long range forces, electrostatic or Van der Waals, are responsible for the twist. We have therefore studied the molecular order in aqueous solution of double helical poly(A)·poly(U) at low ionic strength over a wide concentration range (10–200 mg ml<sup>-1</sup>). In order to avoid the imperfect order due to the entanglements occurring at high molecular weights, we have used relatively short segments. Using a high field magnetic birefringence technique we were able to observe directly and for the first time the transition from the isotropic to the anisotropic phase at a critical concentration (see Results). Furthermore, the local and long range structure of the magnetically aligned sample has been investigated by neutron and light scattering (see Results). Our observations lead us to the conclusion that the anisotropic

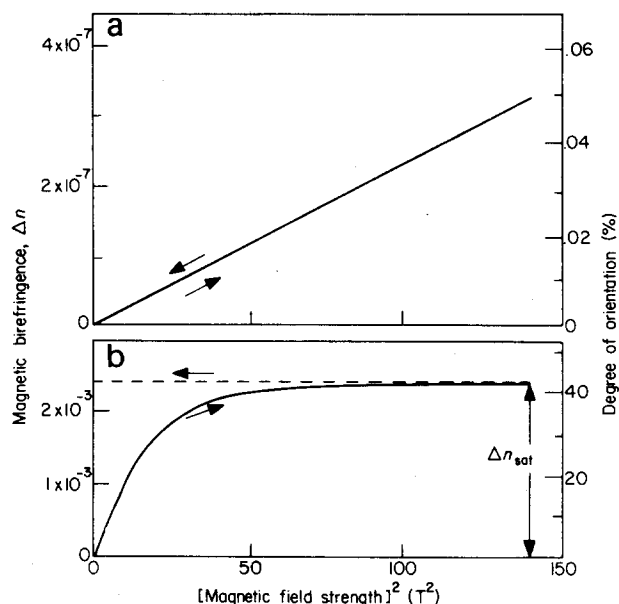
phase may not be of the simple cholesteric type. Instead we propose a three-dimensional tubular structure which seems to be in better agreement with most observations (Discussion). Finally, a simple argument is given in order to show that the observed twist is probably caused by Van der Waals forces (Discussion).

## Experimental

Molecules having an anisotropic diamagnetic susceptibility can be oriented in solution by an external magnetic field,  $H$ . The magnetically induced optical birefringence (Cotton-Mouton effect) is, for small degrees of alignment, given by<sup>22</sup>:  $\Delta n = CM\lambda H^2$ , where  $\Delta n$  is the difference between the refractive indices for light of wavelength  $\lambda$  polarized parallel and perpendicular to  $H$ , and  $CM$  is the Cotton-Mouton constant. For non-interacting molecules,  $CM$  is proportional to the effective molecular anisotropies of the diamagnetic susceptibility  $\Delta\chi$  and optical polarizability  $\Delta\alpha$ , respectively, and the concentration,  $c$ . When intermolecular interaction occurs,  $CM$  is, in addition, proportional to the number of molecules correlated in their mutual orientation. In the case of high molecular weight polymers,  $CM$  is proportional to the persistence length,  $P$ <sup>23,24</sup>. Hence, for liquid-crystal-like ordered solutions or very large stiff rods, the magnetic alignment is very large and  $\Delta n$  saturates at high magnetic fields<sup>25</sup>.

Magnetic birefringences between  $10^{-10}$  and  $\sim 3 \times 10^{-6}$  were measured using a sensitive compensation technique described previously<sup>25</sup>; for larger degrees of birefringence a simpler method could be applied\*. Magnetic fields of up

\* The sample is positioned into a laser beam propagating perpendicular to  $H$ . Crossed polarizers are set at  $-45^\circ$  and  $+45^\circ$  relative to  $H$ . The transmitted light intensity is then proportional to  $\sin^2(\pi\Delta n l/\lambda)$ , where  $l$  is the optical path length. Using photoelastic modulation and lock-in amplifier technique, the measured signal is proportional  $\sin(2\pi\Delta n l/\lambda)$ . During a slow field sweep, maxima and minima of this signal are assigned to field strengths corresponding to  $\Delta n$  values ( $v + 1/4$ ) $\lambda/l$ , and  $(v + 3/4)\lambda/l$ , respectively, for  $v = 0, 1, 2, 3, \dots$



**Figure 1** Magnetic field dependence of the birefringence,  $\Delta n$ , for aqueous solutions of sonicated fragments of poly(A)·poly(U) at concentrations below and above the critical concentration  $c \sim 70 \text{ mg ml}^{-1}$ . (a)  $12 \text{ mg ml}^{-1}$ ; (b)  $98 \text{ mg ml}^{-1}$ . The quantitative degree of orientation  $\Delta n/\Delta n_{\text{max}}$  is derived from the observed birefringence (see footnote in main text). The solutions were characterized by Tris-HCl, pH 7.0. Ionic strength was  $4 \times 10^{-2}$  and  $1 \times 10^{-2} \text{ M NaCl}$  for solutions (a) and (b), respectively. All measurements were performed at room temperature and at  $\lambda = 632.8 \text{ nm}$

to 18.5 tesla were produced by the standard Bitter coils in our laboratory.

The neutron small-angle scattering profiles of magnetically oriented samples were taken in the Institut Laue-Langevin, using the D11 camera<sup>26</sup>. Optical diffraction patterns were either photographed directly from a screen, using laser light of various wavelengths (He-Ne, Ar, Kr), or the angular dependence of the scattered light was monitored by a photomultiplier.

Nucleic acid concentrations were usually measured by u.v. spectroscopy<sup>27</sup>. For a few very concentrated samples they were also determined from the weight of the lyophilized material and the volume of water added. The serial dilution procedure was checked by u.v. spectroscopy on the most dilute sample in a series. Concentrations are given by the mass of solute per volume of the solution, calculated using a solute partial specific volume of  $0.53 \text{ cm}^3 \text{ g}^{-1}$  (Ref. 28). Standard ultracentrifugation techniques were used for the determination of molecular weight.

Poly(A) from Miles Biochemicals ( $s_{20,w}^0 = 8.1$ ) and poly(U) from Miles ( $s_{20,w}^0 = 8.2$ ) and from Serva ( $s_{20,w}^0 = 7.6$ ) were used without further purification. For the preparation of poly(A)·poly(U) dilute equimolar mixtures of poly(A), dissolved in  $\text{H}_2\text{O}$ ;  $0.2 \text{ M NaCl}$ ;  $5 \times 10^{-3} \text{ M Tris}$ , pH 7.4;  $10^{-4} \text{ M EDTA}$ , and poly(U), dissolved in  $\text{H}_2\text{O}$ ;  $0.2 \text{ M Na}_2\text{SO}_4$ , were made. Portions of these solutions were sonicated for several minutes. After a few dialysing and lyophilizing cycles at increasing polymer concentration and decreasing ionic strength (the last dialysis was against  $10^{-3} \text{ M NaCl}$ ,  $10^{-4} \text{ M Tris}$ , pH 7, at  $100 \text{ mg ml}^{-1}$ ), the lyophilized polymer was dissolved in twice-distilled  $\text{H}_2\text{O}$ . The sonicated fragments and high molecular weight polymer showed  $s_{20,w}^0 = 7.5$  and  $12.7$ , corresponding<sup>29</sup> to an average molecular weight of  $3.4 \times 10^5$  and  $2 \times 10^6$  or to a

mean contour length of 170 and 1000 nm ( $\pm 30 \text{ nm}$ ), respectively.

## Results

### Dilute solutions

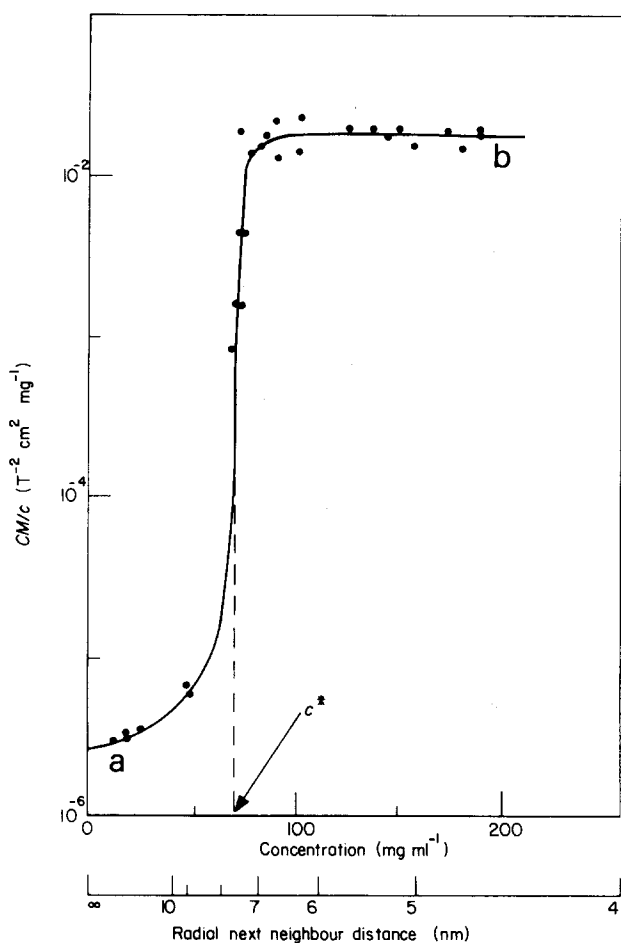
We first describe experiments in the dilute regime, where the persistence length of individual molecules (without interaction with its neighbours) can be observed. This parameter determines the critical concentration at which cooperative ordering sets in, as discussed in the next section. A typical Cotton-Mouton measurement of a dilute solution of segments of poly(A)·poly(U) is shown in Figure 1a. The magnetic birefringence is positive, whereas the intrinsic birefringence of poly(A)·poly(U) with respect to the helical axis is negative<sup>30</sup>. This implies that the double helices tend to orient perpendicular to  $H$ , like DNA<sup>23</sup>. For concentrations well below  $70 \text{ mg ml}^{-1}$  and ionic strengths between  $2 \times 10^{-3}$  and  $4 \times 10^{-2} \text{ M NaCl}$ ,  $\Delta n$  varies linearly with  $H^2$  and linearly with concentration without any detectable hysteresis. A specific Cotton-Mouton constant  $CM/c$  of  $3.1 \times 10^{-6} \text{ T}^{-2} \text{ mg}^{-1} \text{ cm}^2$  is found and is independent of concentration and ionic strength. This value compares well with  $CM/c = 3.0 \times 10^{-6} \text{ T}^{-2} \text{ mg}^{-1} \text{ cm}^2$  for the unsonicated poly(A)·poly(U) sample, as obtained for low concentrations and at similar ionic strengths. Clearly, in both cases there is no noticeable interchain interaction nor is there any indication of a molecular weight dependence of  $CM/c$ . We can therefore apply Wilson's theory<sup>24</sup> of the magnetic birefringence for non-interacting worm-like chains which has been developed for DNA in particular. For the persistence length of the segments we obtain  $P \sim 52 \text{ nm}$ , using<sup>†</sup> for the A-U pair  $\Delta\chi = 9.6 \times 10^{-21} \text{ erg T}^{-2}$ <sup>32</sup>,  $\Delta\alpha = 16.4 \times 10^{-3} \text{ nm}$ <sup>30</sup>,  $M = 660$  and a monomer length of 0.31 nm. In summary, the poly(A)·poly(U) segments have a mean length of 170 nm, which is only a few times larger than the persistence length, and should therefore be considered as relatively stiff chains.

### Concentrated solutions

Above a concentration of  $70 \text{ mg ml}^{-1}$  all poly(A)·poly(U) samples exhibited a multicoloured granulation when observed between crossed polarizers in a polarization microscope; Jizuka made a similar observation<sup>17</sup> on high molecular weight poly(A)·poly(U). From such birefringence patterns, which had already appeared within the first 10 min after preparation of the solution, the mean size of the almost uniformly coloured domains was determined. A mean diameter in the order of a few tens of microns was found. Such domains would include at least  $10^8$  molecules.

Figure 2 shows that around a concentration of  $70 \text{ mg ml}^{-1}$  the Cotton-Mouton constant suddenly increases by almost four orders of magnitude and finally reaches a concentration-independent value of  $\sim 1.8 \times 10^{-2} \text{ T}^{-2} \text{ cm}^2 \text{ mg}^{-1}$ . This demonstrates the onset of intermolecular interactions inducing long range intermolecular order at higher concentrations. When assuming, as a first and simple approach, the existence of independent domains

<sup>†</sup> Since in poly(A)·poly(U) the baseplanes are not exactly perpendicular to the helical axis, but tilted off this direction by an angle of  $13.3^\circ$ <sup>31</sup>, a correction has to be applied reducing  $\Delta\chi$  and  $\Delta\alpha$  of the basepairs by the factor  $(1.5 \cos^2 \theta - 0.5)$ , i.e. by  $\sim 8\%$ .



**Figure 2** Concentration dependence,  $CM/c$ , of the specific Cotton-Mouton constant of aqueous solutions of fragments (mean length 170 nm) of poly(A)·poly(U): (a) and (b) indicate the isotropic and anisotropic phases, respectively. The concentration was varied by serial dilution in twice-distilled  $H_2O$ . The radial next neighbour distances were calculated from the concentration, assuming a hexagonal packing of rods as indicated in Figure 4c and in the text

built up of exactly parallel segments, the change in  $CM/c$  implies that each domain would contain  $\sim 10^3$  molecules and would have a diameter of  $\sim 200$  nm. However, a non-parallel arrangement of the molecules within a domain, e.g. a cholesteric order, would naturally yield a larger diameter. Hence the microscopic and Cotton-Mouton measurements are only in mutual agreement when the poly(A)·poly(U) molecules within a domain are not exactly parallel to each other.

A qualitatively similar conclusion can be drawn from the non-linear behaviour of the birefringence,  $\Delta n$ , in high fields (Figure 1b): Above fields of  $\sim 5$  tesla,  $\Delta n$  saturates towards a value which was found, to increase proportionally with concentration. The reduced birefringence turns out to be  $\Delta n_{sat}/c = 2.1 \pm 0.3 \times 10^{-5} \text{ mg}^{-1} \text{ ml}$ . However, for a solution of poly(A)·poly(U) completely oriented perpendicular to  $H$  one would expect<sup>‡</sup>

‡  $\Delta n_{max}/c$  has been calculated from  $\Delta x$  using the relation:

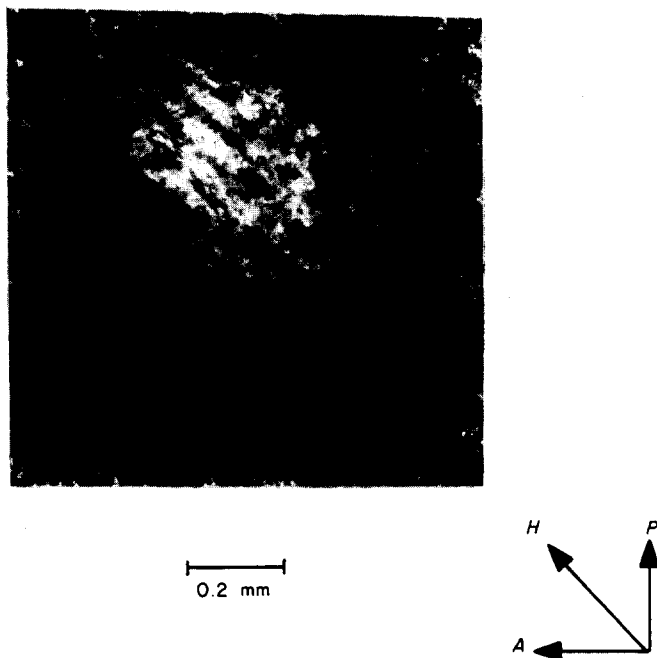
$$\Delta n_{max}/c = \frac{2\pi(n_0^2 + 2)^2}{18n_0} \frac{A_0}{M} \frac{\Delta x}{l}$$

$A_0$  being the Avogadro number,  $M$  the molecular weight,  $n_0$  the mean refractive index of the solution. This relation is easily derived<sup>33,34</sup> from the Lorenz-Lorenz relation for a planar orientation of all molecules.

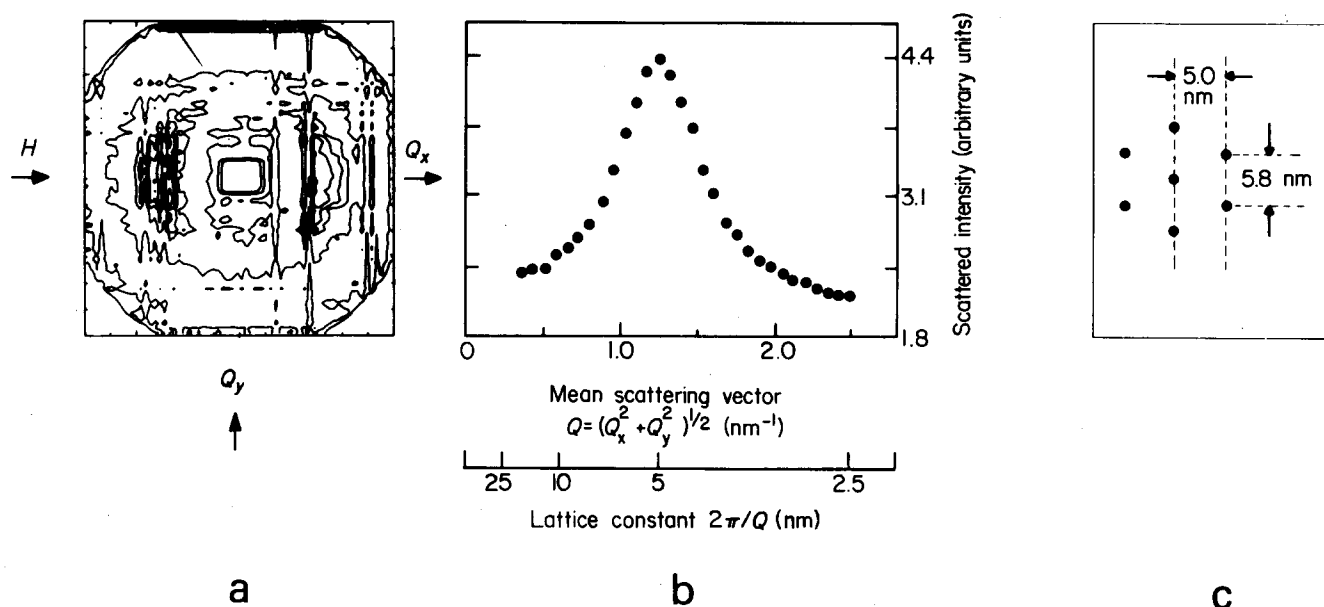
$\Delta n_{max}/c = 5.6 \times 10^{-5} \text{ mg}^{-1} \text{ ml}$ . Hence, less than half of all molecules are oriented in the plane perpendicular to  $H$ , even at fields of around 13 tesla. This might be interpreted either by a 'non-parallel' molecular arrangement within each domain, or by a phase separation of the solution into a strongly anisotropic and isotropic phase.

Figure 1b also shows that  $\Delta n$  maintains its maximum saturation value even when sweeping the magnetic field down to zero. The solution remains strongly oriented for at least a few months and the granular birefringence pattern is unchanged. Only if one moves closer to the critical concentration does the rather regular structure, shown in Figure 3, disappear more rapidly after the orienting field is switched off. As can be seen, 'chains' of homogeneously coloured domains, oriented parallel to  $H$ , are embedded within a disordered but birefringent matrix.

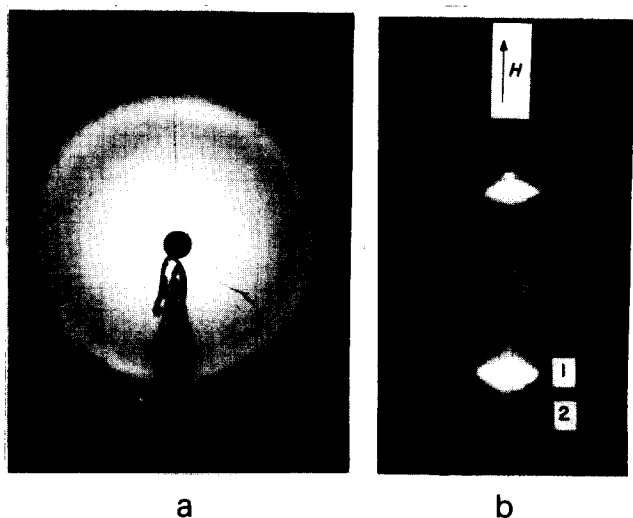
Neutron and light diffraction experiments were carried out in order to obtain more information about the molecular structure. Unless stated otherwise the results agree with the X-ray and light diffraction data reported by Jizuka<sup>17-19</sup>. Typical neutron small-angle diffraction data from a magnetically oriented poly(A)·poly(U) sample are given in Figure 4. A strong peak is observed which corresponds to a Bragg spacing of 5 nm at a concentration,  $c$ , of  $137 \text{ mg ml}^{-1}$  (Figure 4b). This distance is about 2.5 times larger than the diameter of a poly(A)·poly(U) molecule. If one assumes a hexagonal lattice as shown in Figure 4c, this corresponds to a lattice constant of 5 nm, as expected for this concentration if no dilution is allowed along the molecular axes<sup>8</sup>. As can be seen from Figure 4c, the intermolecular distance in this case is 5.8 nm. This short range structure is similar to Luzzati's<sup>35</sup> observation on high molecular weight DNA. Figure 4a demonstrates that the poly(A)·poly(U) molecules are well oriented, with their long axis perpendi-



**Figure 3** Polarization microphotograph of an aqueous solution of fragments of poly(A)·poly(U) at  $c = 75 \text{ mg ml}^{-1}$  taken 1 min after removal from a magnetic field of 13 tesla. The directions of the magnetic field, polarizer and analyser are indicated by the arrows  $H$ ,  $P$  and  $A$ , respectively



**Figure 4** Neutron small-angle scattering data of an aqueous solution of sonicated fragments of poly(A)·poly(U) after removal from a magnetic field of 18.6 tesla;  $c = 137 \pm 15$  mg ml<sup>-1</sup>. (a) Two-dimensional  $Q_x$ ,  $Q_y$  contour plot of the scattered intensity. Lines connect points of constant scattered intensity. The field direction is horizontal. (b) Scattering intensity as a function of the mean scattering vector (averaged between  $Q_x$  and  $Q_y$ );  $\lambda_{\text{neutron}} = 0.515$  nm. Background scattering has been subtracted. The strongest scattering corresponds to a lattice constant of 5 nm. (c) Hexagonal lattice



**Figure 5** Light diffraction pattern of an aqueous solution of sonicated poly(A)·poly(U);  $c = 190 \pm 10$  mg ml<sup>-1</sup>. (a) Before orientation and (b) after orientation by a vertical magnetic field of 13 tesla, but sample removed from the magnet. (1)  $\lambda = 482.5$  nm, (blue); (2)  $\lambda = 632.8$  nm (red)

cular to  $H$ , in agreement with our birefringence data above.

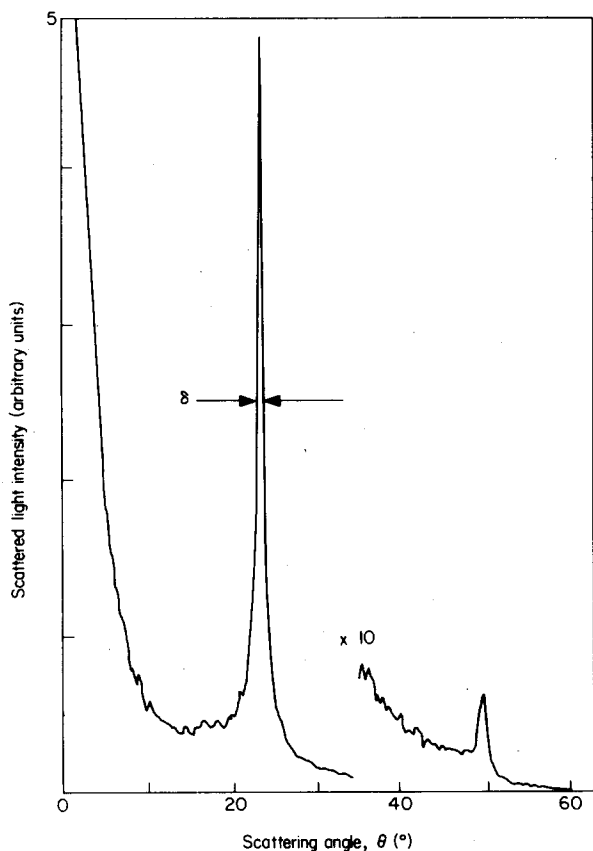
When samples in the anisotropic phase are illuminated with white light, they display various colours within minutes of preparation, which depend on the angle of observation. When illuminated with a laser beam, a diffraction ring is observed for unoriented samples (Figure 5a) and relatively sharp arcs in the direction of  $H$  for oriented samples (Figure 5b). Figure 6 shows the angular dependence of the scattered light intensity. Besides a strong scattering at very low angles, which is probably due to the average size of the domains, the diffraction is characterized by a very sharp peak at a

scattering angle  $\theta = 23.8^\circ$  and a second much weaker maximum at  $\sim 48.8^\circ$ . This corresponds to a superstructure having a periodicity of 1250 nm. The extremely small angular width,  $\delta$ , of the order of  $1^\circ$  indicates that this superstructure coherently extends over at least 10 periods, which might be the size of a birefringent domain ( $\sim 20$   $\mu\text{m}$ ). Most interestingly, the sharpness of the diffraction line is apparently not disturbed by the broad molecular weight distribution in the sonicated samples. The concentration dependence of this long range period is given in Figure 8. It can be seen that the period varies as  $c^{-0.5}$ . Similar optical diffraction has also been reported by Jizuka<sup>19</sup> to appear in solutions of high molecular weight poly(A)·poly(U), but only after several months. The structural order in these solutions of high molecular weight seems to be less pronounced, when compared with ours, probably due to the long time needed for the very long polymer chains to reach their ordered state. Jizuka's finding of a concentration dependence of the optical periodicity ( $\sim c^{-1.1}$ ) does not agree with our data (Figure 8), and at present we do not have any explanation for this lack of agreement. Cotton-Mouton, birefringence and light diffraction data almost identical to those reported above have also been found on DNA-molecules all having one well defined length of only 50 nm.

## Discussion

### Structure of the concentrated solutions

The optically observed superstructure and the circular dichroism in the visible spectrum in concentrated solutions of poly(A)·poly(U) and DNA have been interpreted by the existence of a simple cholesteric texture<sup>15,17</sup>. It was thereby assumed that the rods lie exactly parallel to each other within planes, but that there is a small twist angle between molecules in adjacent planes.



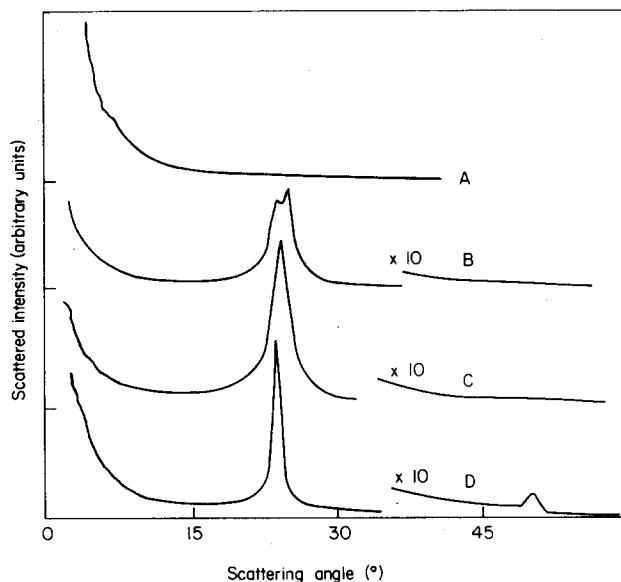
**Figure 6** Angular dependence of the scattered light intensity from a solution of magnetically oriented poly(A)·poly(U) molecules in the anisotropic phase ( $c = 137 \pm 15 \text{ mg ml}^{-1}$ ,  $\sim 4 \times 10^{-3} \text{ M NaCl}$ ). The sample cell had an optical path of 1 mm. The instrumental angular resolution was  $1^\circ$ .  $\lambda_{\text{opt}} = 514.5 \text{ nm}$

Cholesteric structures have indeed been found in very dense solvent-free phases of DNA, for example, in chromosomes of algae<sup>21</sup> and in the so-called  $\psi$ -condensates<sup>36</sup>. In contrast to the above interpretation<sup>15,17</sup>, we found that our solutions of segments of poly(A)·poly(U) and of DNA are probably not in a simple cholesteric phase. This conclusion is based on the fact that less than half of all the molecules are aligned perpendicular to the magnetic field, even in fields of  $\sim 13$  tesla, where the birefringence and the degree of orientation have already reached their final value (see Results and Figure 1b).

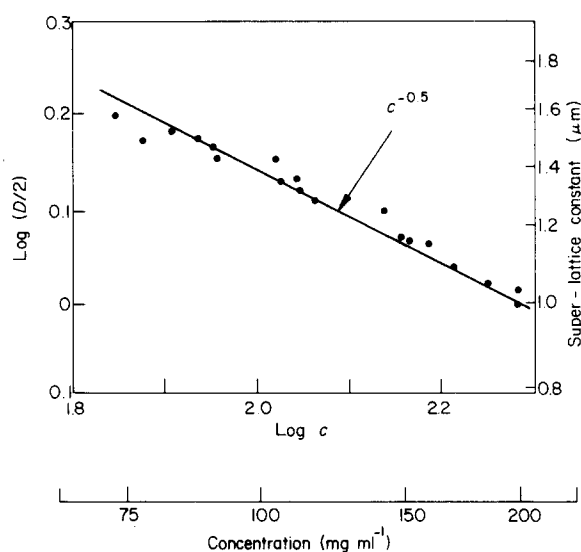
We now propose a possible model for the three-dimensional spatial arrangement of rod-like helices which is qualitatively consistent with our observations. We start from a central helix (Figure 9) along the  $z$ -axis, oriented normal to the plane of the Figure. Propagating in radial direction  $r$  we allow a constant twist angle,  $\theta = 2\pi r/D$ , between neighbouring helices and obtain a concentric tubular structure with radial periodicity  $D/2$ . For simplicity we assume translational symmetry in the  $z$ -direction. How would such a structure orient in a magnetic field and what would be its maximum birefringence? From geometrical arguments based on Figure 9, one can show that roughly half of all  $N$  molecules are perpendicular to the  $z$ -axis, while  $3N/4$  are at right angles to  $x$ . The energy in the magnetic field can be minimized when the whole tubular structure orients perpendicular to the field, because in this case the number of molecules oriented perpendicular to  $H$  exceeds (by  $N/4$ ) those

oriented parallel to  $H$ . The maximum birefringence is reduced (by a factor 4) relative to a fully oriented simple cholesteric phase. Clearly, quantitative agreement with our magnetic birefringence measurements cannot be expected, since all defects have been neglected. The reported neutron and light diffraction pattern agree with this model, but the long-range packing of the cylinders is ignored here.

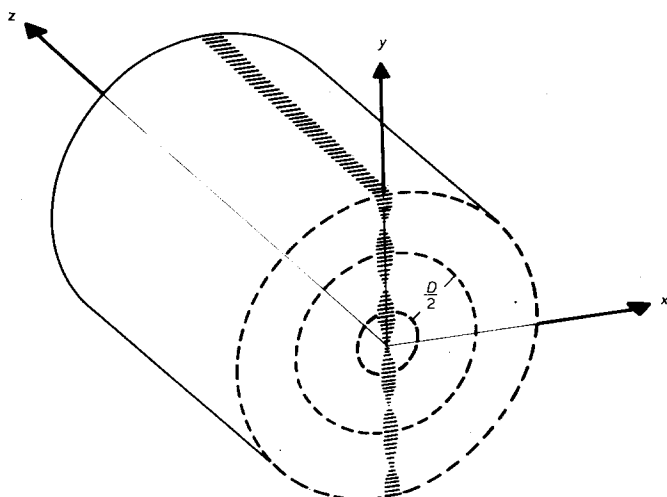
A tubular structure proposed here may be closely related to the circular structures in polypeptide solutions which Robinson *et al.*<sup>8</sup> observed directly by means of a microscope (see Figure 3 of Ref. 8) and which he called 'spherulites'.



**Figure 7** Scattering intensity vs. angle for various increasing temperatures (see Figure 6 for other details).  $\lambda_{\text{opt}} = 514.5 \text{ nm}$ . A, 58.5°C; B, 35°C; C, 30°C; D, 22°C. One can clearly distinguish the melting of the superstructure (occurring at around 47°C). On cooling, an unoriented superstructure reappears



**Figure 8** Concentration dependence of the period of the super-lattice in solutions of poly(A)·poly(U) molecules, as obtained from optical diffraction on samples specified in the caption of Figure 2. The period corresponds to half the cholesteric period,  $D$  (see Figure 9)



**Figure 9** A possible model for the spatial arrangement of helical molecules. Solid lines indicate projections of the molecular helical axis onto the  $x$ - $y$ -plane. Translational invariance is assumed in the  $z$ -direction

#### Long-range intermolecular forces

Here we discuss first the forces which are responsible for the sudden onset of nearly parallel order when a critical nucleic acid concentration is surpassed. For a theoretical treatment of hard-core, electrostatic and Van der Waals interactions between rod-like charged particles the reader is referred to recent work by Parsegian, Brenner, McQuarrie and Ninham<sup>37-43</sup>, and by de Gennes *et al.*<sup>44</sup>, Oosawa<sup>45</sup>, and Kirkwood *et al.*<sup>46</sup>.

Considering only *hard core repulsion*, Onsager<sup>47</sup> first predicted that a solution of rods with length  $L$  and diameter  $a$  should for entropic reasons undergo a transition from an isotropic phase to a partially nematic state at a critical concentration  $c = 13.4/\pi L^2 a$ . Using, to a crude approximation, the persistence length (52 nm) as an effective length over which the poly(A)·poly(U) molecules can be considered as rigid rods, we obtain (with  $a = 2$  nm)  $c^* = 149$  mg ml<sup>-1</sup>, compared with the experimental value  $c^* \sim 70$  mg ml<sup>-1</sup>. It seems evident that in our case of highly charged molecules more long range interactions are responsible for the observed order.

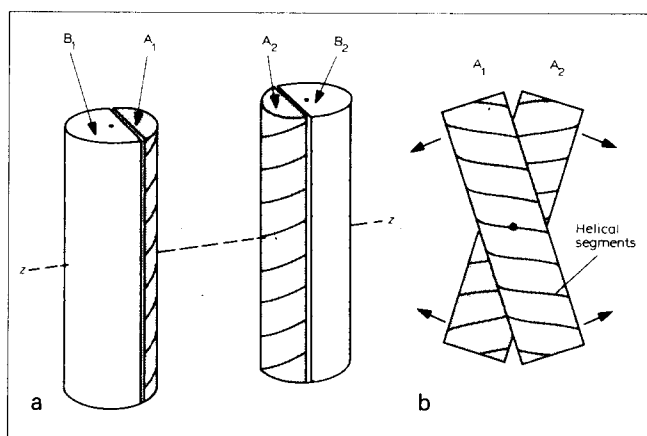
Recently, Stigter<sup>48</sup> extended Onsager's theory by including *electrostatic interaction* and applied it to rigid DNA rods in electrolytic solutions. He replaced in Onsager's condition for  $c^*$  of rod diameter  $a$  by a larger effective diameter,  $a_B$ , which is related to the diameter of the counterion cloud surrounding the highly charged DNA molecule and is therefore strongly dependent on the external salt concentration. Values for  $a_B$  range from 2.95 nm (at 1 M NaCl) to 22.3 nm (at 0.05 M NaCl). His theory is only valid for long rods ( $L/a_B \geq 10$ ). It can be applied to our case only if either the flexibility of the chain is disregarded ( $L = 170$  nm and  $a_B \sim 16$  nm at the experimental ionic strength of 0.01 M NaCl,  $L/a_B \sim 10$ ) or if an unrealistically high ionic strength of 10<sup>-1</sup> M NaCl,  $a_B \sim 5.6$  nm at the correct persistence length  $P = 52$  nm is used ( $P/a_B \approx 10$ ). One then obtains from Stigter's theory a critical concentration  $c^*$  of 6 and 56 mg ml<sup>-1</sup>, respectively. The first  $c^*$  value is expected to increase when corrected for flexibility, whereas the second should decrease when corrected for the experimental ionic strength. This implies that the theory should be extended to fewer anisotropic cases ( $P/a_B < 10$ ) in order to cover the interesting experimental conditions found in many polyelectrolytes. It also

demonstrates that electrostatic repulsion plays an important role in parallel ordering.

As has been clearly stated<sup>37-40</sup>, the *Van der Waals interaction* between rod-like charged particles might also become important, particularly for large separations between the particles. Due to ionic screening the electrostatic repulsive force decays exponentially with distance from the charged particle. On the other hand, the attractive Van der Waals force dies out more slowly and follows a constant power law. Consequently, at a certain sufficiently large distance there should be a balance of both forces, resulting in a binding energy of two parallel rod-like particles. Naturally the binding energy increases with the length of the rods. For example, Parsegian<sup>39</sup> has estimated that two parallel linear chain molecules having a diameter of 2 nm each and a mutual distance of 5 nm would require a minimum length of 1500 nm for the Van der Waals binding energy to exceed  $kT$ . Since our molecules have such a diameter and a tenth of this length ( $\sim 170$  nm) the Van der Waals force, although weaker than the Coulomb-repulsion by perhaps one order of magnitude, is not negligible. In addition, an 'ionic' Van der Waals attraction due to counterion fluctuations has been predicted<sup>45</sup> to become very important at small separations (1 nm in our case).

In fact, it seems that only the Van der Waals forces are responsible for the small, but well defined, twist angle between neighbouring molecules. From pure symmetry considerations it seems plausible that the twist between neighbouring molecules may be related to the helical structure of each molecule. At the large interparticle distance with which we are dealing here, each molecule probably undergoes a free thermal rotation around its rod-axis and axial motion. Therefore, it is hard to imagine how electrostatic forces between neighbouring molecules could induce a twist, whatever the charge distribution on each molecule. Goosens<sup>49</sup> and Samulski *et al.*<sup>50</sup> were the first to describe how the helical anisotropy of the polarizability of each molecule could lead to a twisting Van der Waals force. Unfortunately their theory does not result in any twist if each molecule can rotate freely about its rod-axis, as is probably the case here (particularly for the short segments we used).

In our view this limitation is not serious. As we will show by the following qualitative argument, the Van der Waals interaction leads to a tilting force between helical molecules even if they rotate. Let us, for example, consider two helical molecules shown in *Figure 10a* and let us cut each of them into two vertical sections, A and B. The shaded sections  $A_1$  and  $A_2$  are closest to each other and therefore their Van der Waals interaction ( $A_1, A_2$ ) is strongest (stronger than between the other pairs,  $A_1 B_2$ ,  $A_2 B_1$  and  $B_1 B_2$ ). Therefore, in *Figure 10b*, we have considered only the two sections  $A_1$  and  $A_2$  of *Figure 10a* as seen along the  $z$ - $z$  axis passing at right angles through both molecules. Let us assume that the maximum polarizability of each section is parallel (or perpendicular) to its helical segments. Since, in general, Van der Waals interaction between two anisotropic molecules tends to align their axes of maximum polarizability parallel to one another, a torque (indicated in *Figure 10b*) would arise, tending to orient the helical segments of both sections parallel to each other, thereby creating the tilt between both molecules. The mechanism described here remains qualitatively fully active whether or not the molecules are in a state of thermal rotation around their axis.



**Figure 10** (a) Van der Waals interaction between two almost parallel helical molecules is mainly determined by the shaded cylindrical sections  $A_1$  and  $A_2$ , which face each other. Sections  $B$  are more remote from one another and thus the Van der Waals force between them is smaller. (b) The two shaded sections  $A_1$  and  $A_2$  of (a) seen along the  $z$ - $z$  axis. A tilting Van der Waals force tends to align the helical segments of  $A_1$  with those of  $A_2$ , thus trying to orient the direction of maximum polarizability parallel to each other

### Acknowledgements

We thank M. Leng for advice during the preparation of poly(A)·poly(U) fragments performed in his laboratory at the Institut de Biophysique Moléculaire, Orleans. The neutron scattering experiments were carried out by J. Torbet at the Institut Laue-Langevin, Grenoble and light scattering measurements by R. Oldenbourg. We are very grateful to both for their important contributions to this work. We acknowledge the help of the staff of the Service National des Champs Intenses, Grenoble, where the Cotton-Mouton measurements were performed, and we thank M. Bichler for his permanent technical support. We are also indebted to Mme Rinaudo for her continual interest, to G. Eilenberger, R. Klein and H. Schroeder for stimulating and clarifying discussions.

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