Molecular Imaging of Tobacco Mosaic Virus Lyotropic Nematic Phases

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Aqueous solutions of tobacco mosaic virus are ideal systems for visualization of "molecular" distributions in the nematic phase by freeze-fracture electron microscopy. The order parameter in well aligned regions is measured to be greater than 0.9. Twist deformations appear to be most common, confirming that the twist elastic constant is smaller than either the splay or bend constant. Edge and screw disclinations are observed in tobacco mosaic virus nematics in configurations predicted by continuum theory. Disclination cores have been observed for the first time.

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Both colloidal-crystal and nematic liquid-crystal phases are formed by tobacco mosaic virus (TMV) in water, depending on the virus concentration, ionic strength, and pH.[1] The virus particles, each a rigid rod 320 nm long and 20 nm in diameter, are the structural equivalents of molecules in the more typical solid and thermotropic liquid crystals. The 2 orders-of-magnitude size increase between TMV and molecular solid and liquid crystals allows the "molecular" order in TMV solutions to be seen and interpreted by means of freeze-fracture replication transmission electron microscopy.

The nematic virus dispersions examined here were extracted from infected tobacco plants at Brandeis University by techniques previously described.[1] A 0.1–0.5-μl drop of the nematic was trapped between two cooper planchettes (Balzers, Hudson, New Hampshire) to form a 10–50 μm-thick layer of sample. The sample sandwiches were annealed for 3–5 min in a humidity-controlled glovebox to allow the virus to equilibrate and remove any effects of flow alignment. The samples were then rapidly frozen (> 15 000 °C/sec) in the opposed high-velocity jets of liquid propane at $-180\,^{\circ}$C in a Balzers cryojet apparatus. At these cooling rates, water is apparently vitrified; no evidence of ice-crystal formation is observed and the virus distribution is preserved without disruption.[2] The jet-frozen specimens were fractured at $-170\,^{\circ}$C and 10^{-8} Torr in a Balzers 400 freeze-etch apparatus. The fracture surfaces were replicated with 1.5 nm of platinum evaporated at a 45° angle and 15 nm of carbon deposited normal to the fracture surface.

Images of freeze-fracture replicas of TMV nematics show that the virus particles are well ordered over many virus lengths (Fig. 1). Many of the virus particles cross fracture through their centers, and show the 4-nm-diam central hole down the axis of the virus. The fine striations normal to the virus axis visible in the micrographs are the helical arrays of individual protein subunits that make up the outer shell of the virus.[1] An estimate of the order parameter, S, of the TMV nematic was obtained by our measuring the projected length of the virus along the average orientation (N in the micrograph), then dividing by the total length of the virus. This gives $\cos\theta$, in which θ is the angle of deviation of an individual virus from the average direction. The order parameter is defined to be[3]

$$S = \tfrac{1}{2}\langle 3\cos^2\theta - 1\rangle .$$

S is measured to be 0.93, in good agreement with the 0.9 measured by preliminary x-ray studies.[4] As expected, the order parameter measured over small areas is greater than the bulk value measured by x ray.

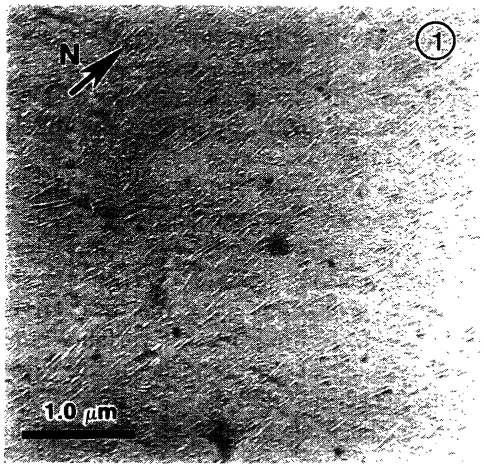

FIG. 1. TMV nematic of virus particles of average length 320 ± 17 nm. N is the director. The order parameter is 0.93.

However, because any artifacts of freezing, such as thermal gradients or ice-crystal formation, tend to decrease the order, the agreement between the two methods shows that the freezing rate is high enough to prevent the virus from rearranging.

Distortions in the direction of local molecular orientation in TMV nematics require little energy, as in thermotropic nematics. All distortions in a nematic phase are the sum of three principal modes: splay, twist, and bend (Fig. 2), each with its associated elastic constant.[5] For rigid-rod nematics such as TMV, the bend elastic constant is predicted to be large in comparison to the splay and twist constants.[6,7] Experimentally, K_2, the twist constant, is of the order 10^{-7} dyn/cm; K_1, the splay constant, is about 5 times as large; and K_3, the bend constant, is about 40 times K_2.[8] In freeze-fracture images, twist deformations are most common and are manifested as a rotation of the virus particles out of the fracture plane. Twist deformations seem to occur more often and over a shorter distance than either splay or bend deformations, which suggests that the twist elastic constant is smallest. In splay and bend deformations, the virus particles remain in the plane of the director.

Disclinations, around which the molecular orientation changes discontinuously, are well described by singular solutions of the continuum equations.[5] Two types of disclinations exist: edge (or twist) disclinations [see Fig. 3(a)] which have their rotation axis perpendicular to the disclination line,[3] and screw (or wedge) disclinations which have their rotation axis parallel to the disclination line [see Fig. 3(b)]. However, the continuum equations cannot describe the molecular configuration near the core of the defect,[3,5] an understanding of which is essential to theories of the blue phases of cholesteric liquid crystals.[9]

Figure 4(a) shows a TMV nematic sample fractured near a $|\frac{1}{2}|$ edge disclination line. The virus particles abruptly change orientation by 90° along the disclination line, similar to the drawing in Fig. 3(a). The disclination core is narrow, never appearing to be more than one virus particle in width. There appear to be small pockets of water (arrowed) along the core, possibly to fill in any gaps in the virus arrangements, thereby relieving any regions of large stress. The thermotropic nematic analog to the patches of water may be local variations in density at the disclination core; however, the virus distribution does not appear to be isotropic near the core as is commonly assumed.[9]

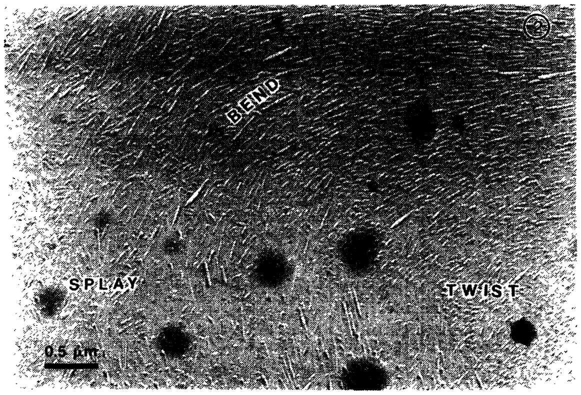

FIG. 2. Three principal modes of deformation: bend, twist, and splay.

Figure 4(b) shows a $-\frac{1}{2}$ screw disclination line almost perpendicular to the fracture plane. The configuration around the line is similar to Fig. 3(b). However, near the core of the disclination, the virus parti-

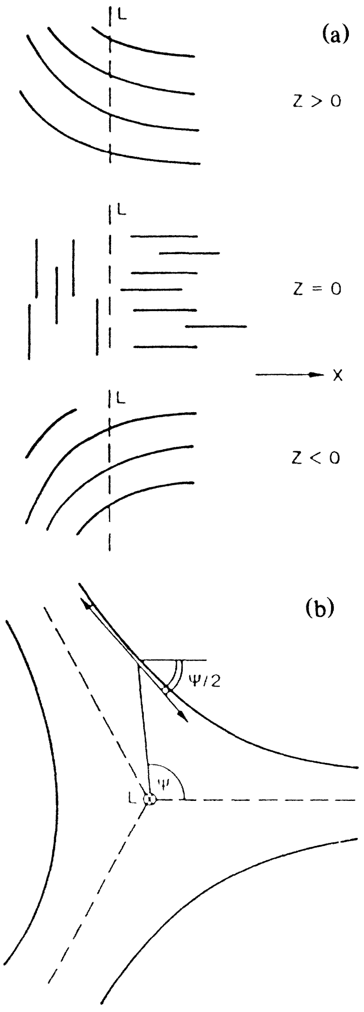

FIG. 3. (a) Molecular arrangement in successive horizontal planes around a $|\frac{1}{2}|$ edge disclination. (b) Geometrical construction and molecular arrangement of a $-\frac{1}{2}$ screw disclination; L is the disclination line as well as the rotation axis.

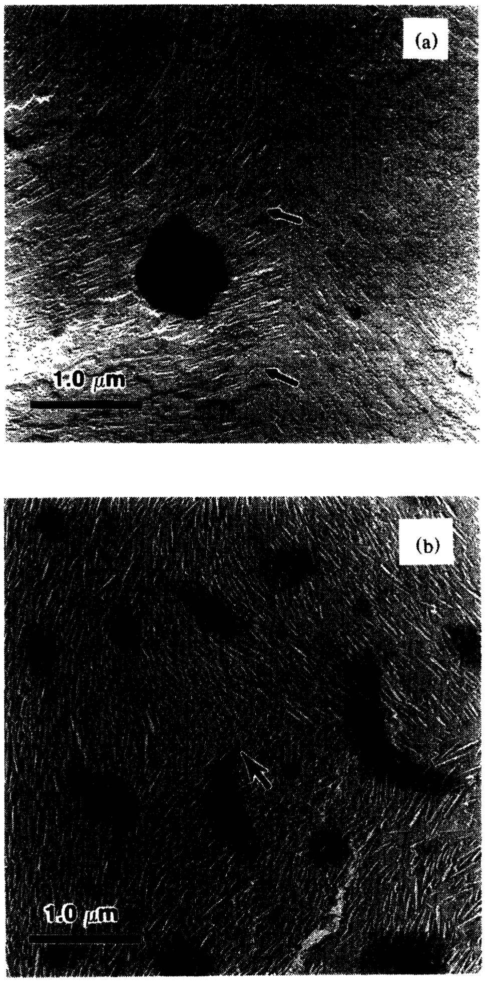

FIG. 4. (a) Twist disclination in a TMV nematic. The virus particles abruptly rotate by about 90° along the disclination line. The fracture plane is tilted slightly from the plane of the disclination so that the rotation angle is somewhat less than 90° in the center of the figure. Small pockets of water (arrows) seem to fill in the gaps along the core, which is no more than one virus particle wide. Compare to Fig. 3(a). (b) $-\frac{1}{2}$ screw disclination in a TMV nematic. The core of the screw disclination appears larger than in the edge disclination and is also more disordered. The virus particles appear to twist out of the fracture plane along the disclination line (arrow). Compare to Fig. 3(b).

cles appear much more disordered and seem to twist out of the fracture plane (arrows). The core of the screw disclination is several virus particles wide. The deformation around a screw disclination line is mainly splay and bend, which are higher-energy processes, as compared to an edge disclination, which involves mainly twist. As the energy of a disclination increases with the size of the core, Figs. 4(a) and 4(b) suggest that edge disclinations are of lower energy than screw disclinations in TMV nematics, and hence would be more numerous. In bulk samples of TMV observed in the light microscope, edge disclination loops are the predominant defects.[1]

These freeze-fracture images present the first real-space view of molecular order in a nematic liquid crystal. In future experiments, the perturbing influence of the fracture process may be eliminated by sublimation of several nanometers of ice from the fracture surface prior to replication to reveal the virus distribution underlying the fracture plane. By digitization and analysis of the micrographs, quantitative information including order parameters, autocorrelation functions, and radial distribution functions for the nematic TMV solutions might be obtained.[2]

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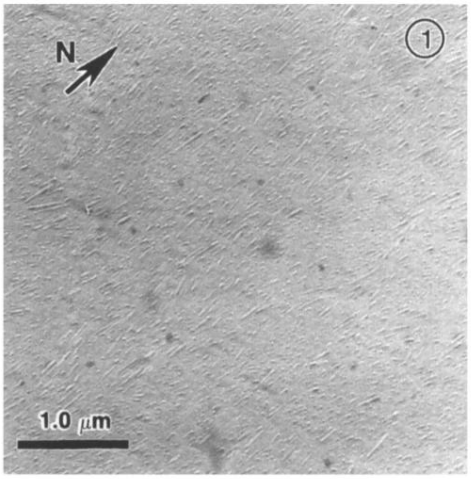

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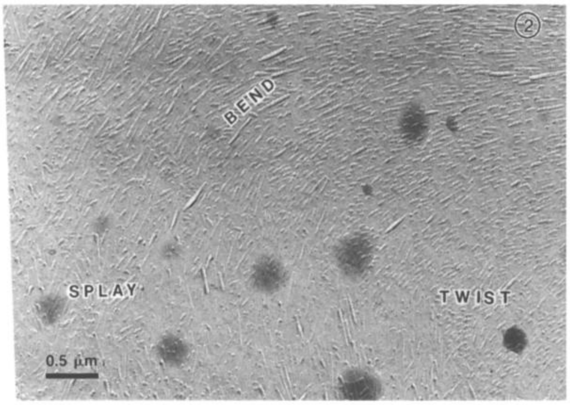

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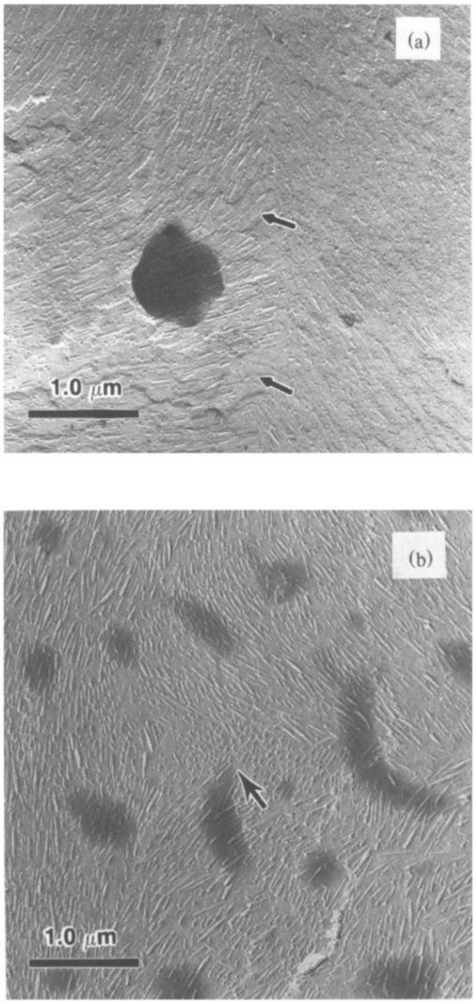

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