

Fibre diffraction and diversity in filamentous plant virus structure

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Fibre diffraction has a long history of successful structural analysis of tobamoviruses such as tobacco mosaic virus. Studies of other filamentous plant viruses, however, have been fraught with difficulties. These difficulties stem primarily from problems of specimen preparation rather than the inherent complexity of the viral structures. Low yield, low solubility, and flexibility have all contributed to these problems. Nevertheless, over the years diffraction patterns have been obtained from members of several filamentous plant virus groups, and the potexviruses in particular are beginning to provide high quality fibre diffraction data.

Tobacco mosaic virus

Filamentous plant viruses are among the oldest fibre diffraction specimens; the first diffraction patterns from tobacco mosaic virus (TMV) were described in 1936 [1], and TMV has served as a model for the development of fibre diffraction methods ever since then.

Techniques for making oriented sols of TMV were originally developed by Bernal and Fankuchen [2] and refined by Gregory and Holmes [3]. These sols were made by drawing virus from a centrifuged pellet into an X-ray capillary tube, and moving the column of virus, usually mixed with a little buffer solution, back and forth in the capillary to induce orientation by shearing forces. Sols made in this way had concentrations of 200 to 300 mg/ml, and disorientation angles of little more than 1° , and they are still among the best diffracting fibre specimens in biology. In fact, one such sol made by Holmes in 1960 was the source of diffraction patterns taken in 1982 and used in the structure determination of TMV at 2.9 Å resolution [4].

TMV also served as a model for fibre diffraction data processing, particularly using the method of angular deconvolution [5], and for objective methods of phase determination. Isomorphous replacement was

used very early to obtain radial density distributions of TMV [6,7], and developed further to solve the multi-dimensional phase problem in high resolution structure determination by fibre diffraction [8]. Other techniques developed or refined using TMV included layer line splitting [9] and molecular replacement [10]. Methods of structure refinement and evaluation were also developed using TMV; examples include restrained least-squares refinement [11], molecular dynamics refinement [12], difference Fourier analysis for fibre diffraction [13] and fibre diffraction *R*-factors [14,15].

These methods were used to determine the structure of TMV at 2.9 Å resolution, and to refine it to an *R*-factor of 0.096 [4]. Related virus structures followed. The structure of the U2 strain of TMV (U2) was determined at 3.5 Å resolution by molecular replacement from TMV [10], the structure of cucumber green mottle mosaic virus (CGMMV) was determined at 3.4 Å resolution using a combination of isomorphous replacement and molecular replacement [16], and the structure of ribgrass mosaic virus (RMV) was determined at 2.9 Å resolution by molecular replacement [17]. These three virus structures were all refined by molecular dynamics methods [12]. The RMV structure is probably the best-determined tobamovirus structure; its *R*-factor is 0.095, very close to that of TMV, but the geometry of the RMV model is considerably better.

These structure determinations are all very encouraging for those who use fibre diffraction methods. They are the largest structures to be determined in atomic detail by fibre diffraction, and they have been of considerable use in interpreting the chemistry and biology of the viruses. But the coat proteins of the viruses are highly homologous. TMV and CGMMV are 36% identical, while TMV and RMV are 46% identical. And although there are important differences among the structures, the protein folds are extremely similar (Figure 1).

Other filamentous plant viruses

Despite successes with the tobamoviruses, there has been little progress in fibre diffraction from other filamentous plant viruses.

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Figure 1: The coat proteins and nucleic acid binding sites of three tobamoviruses. Coat protein folds are represented by ribbon diagrams; RNA by skeletal models. (a) TMV (b) CGMMV (c) RMV.

Viruses currently recognizes 22 genera of plant viruses [18]. A few are morphologically similar to each other at the electron microscopic level, but most exhibit large differences in both morphology and chemical structure. Some authors have suggested that the protein structures of all filamentous plant viruses are similar. A better argument has been made that most fall into one of two groups, the rigid rods or the flexible filaments [19]. Arguments have been made from protein sequence information that the tobamoviruses, the tobnaviruses, and the furoviruses share a common protein fold, the four α -helix bundle [20,21]. But although this postulate is probably true, the amino acid sequences and protein chain lengths of viruses in different groups are so different that there must be major differences in the structures, too great to predict without specific structure determinations. And, as with most filaments, those structures can only be determined satisfactorily by fibre diffraction.

Good diffraction patterns have been obtained from a few viruses. In 1965, Finch [22] published patterns from the tobnavirus pepper ringspot virus (at that time called tobacco rattle virus, CAM strain) and the hordeivirus barley stripe mosaic virus. The diffracting specimens were made by the method of Gregory and Holmes [3]. Diffraction patterns from dried fibres were obtained for a number of potexviruses, including potato virus X (PVX) [23] and papaya mosaic virus (PMV) [24], by Tollin and his colleagues. Most of the patterns were not good enough for high resolution structure determination, however, and none were used for more than the determination of the helical symmetry and, in one case [22], an estimate of a radial density distribution. None of the investigations was continued, and there

has been little or nothing published in this field for the past twenty years. Nevertheless, there continues to be great interest in these viruses, particularly in the potexviruses. Potexviruses can accommodate large insertions in their coat proteins [25], and consequently have great potential for large-scale, inexpensive production of vaccines and other therapeutically and biotechnologically useful peptides. Designing suitable insertions, however, requires knowledge of the viral structure.

This topic should not be left without noting that there have been successful studies of the filamentous bacteriophages. All of the bacteriophages studied have simple, highly α -helical protein structures and relatively small coat proteins, so structures could be determined by model building and refinement [26]. Dried fibre samples were oriented in strong magnetic fields, and the most recent structures [27,28] have been refined using molecular dynamics methods [12].

Problems and solutions

Why are the tobamoviruses such ideal subjects for fibre diffraction analysis, while other filamentous plant viruses are so obdurate?

Despite the many years that were required and the great difficulties that had to be overcome to determine its structure, TMV is an unusually investigator-friendly virus. It is easy to grow (yields of 1 g from 1 Kg of tissue are not unusual), exceptionally stable (we have samples in our laboratory that are decades old), very soluble, and relatively insensitive to radiation. It is a rigid rod, and its aspect ratio of 15 appears to be close to ideal

| | symmetry (u/t) (subunits/turns) | radius(Å)(r) | r/u |
|--------------|------------------------------------|--------------|------|
| TMV | 49/3 | 90 | 1.8 |
| PVX | 35/4 | 65 | 1.8 |
| TRV | 76/3 | 110 | 1.4 |
| Pf1 | 27/5 | 30 | 1.1 |
| microtubules | 13/4 | 150 | 11.5 |

Table 1: Intensity overlap (approximately r/u) induced by cylindrical averaging in helical assemblies. Symmetries of TRV and PVX are best estimates.

for orientation. But the flexible filamentous bacteriophage Pf1 has an aspect ratio of 300. Large aspect ratios and flexibility are not insuperable obstacles to orientation. Furthermore, aspect ratios can be modified by shearing or genetic modification if necessary. Flexibility is more difficult to control, although it may be altered by solution conditions, genetic modification, or magnetic orientation.

Namba's group has had considerable success orienting bacterial flagella for fibre diffraction using a combination of centrifugation and magnetic orientation [29,30]. In some cases, they have achieved orientations as good as 0.6° . It seems reasonable, given the morphological similarities between flagella and some of the flexible filamentous plant viruses, to hope that viruses might also respond to these treatments.

Symmetry is not a limitation in fibre diffraction of plant viruses. The degree of intensity overlap induced by cylindrical averaging is well approximated by the ratio of the filament radius to the number of subunits in the helical repeat. This

ratio (Table 1) does not vary greatly among viruses, in contrast with some other filamentous assemblies.

The problems in working with filamentous viruses are related either to small yields of viruses or to biochemical problems such as aggregation, which in turn are usually related to surface charge. We have had some limited success modifying surface charge chemically, and we have also made some simple genetic modifications (for example, removing short terminal segments from the coat protein chain). Nevertheless, at this time, the best candidates for fibre diffraction analysis are still those viruses that can be produced in relatively large quantities, and are highly soluble. These criteria suggest that after the tobamoviruses, the best fibre diffraction specimens should be the potexviruses.

Recent work with potexviruses

We have directed our efforts toward developing PVX and PMV as fibre diffraction subjects. Both of these viruses are available in high yields, and both are very soluble.

PMV was purified from infected papaya trees using a protocol adapted from that of Erickson and Bancroft [31]. The modifications included the use of protease inhibitors and reducing agents. PVX was purified from infected tobacco plants (*N. clevelandii*) using a procedure modified from that of Goodman [32]. Again, protease inhibitors and reducing agents were used. Details of these purifications will be published elsewhere.

Sols were drawn into 0.5 mm glass capillaries from soft pellets that had been centrifuged for 24 hours at

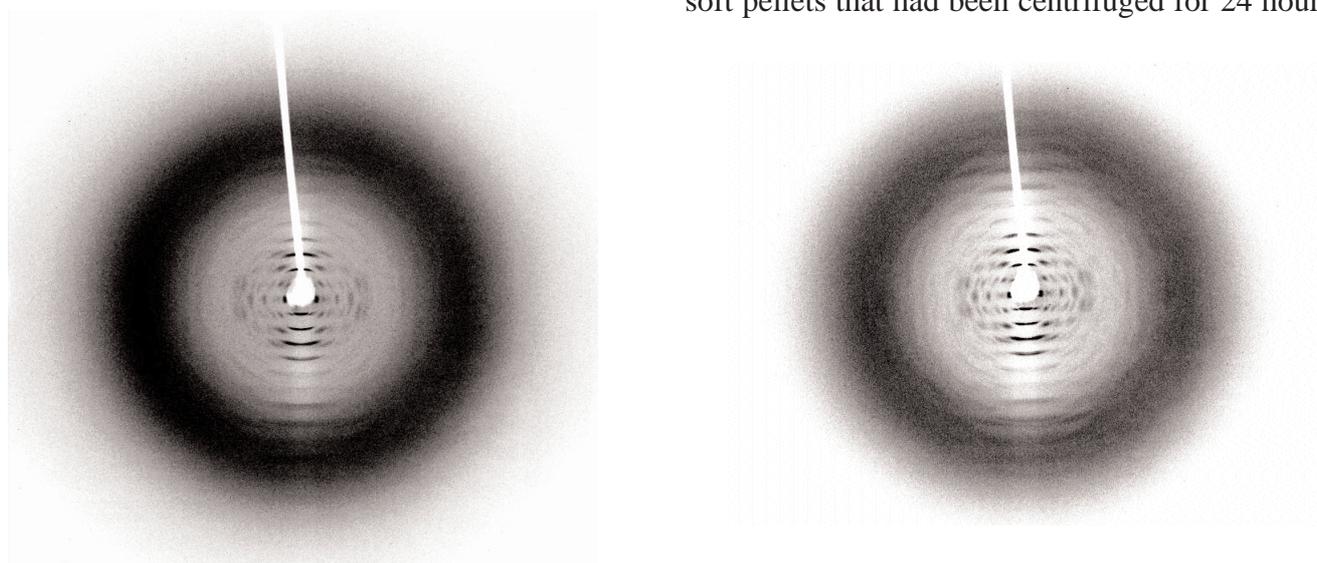


Figure 2: Diffraction patterns from magnetically oriented sols of (a) papaya mosaic virus and (b) potato virus X.

11,000 g. The ends of the capillaries were sealed, and the capillaries were centrifuged for 72 hours at 2000 g in a swinging bucket rotor, following procedures similar to those of Yamashita, Suzuki, and Namba [30]. The dilute regions at the top of the sols were removed using a smaller capillary, and the capillaries containing the sols were sealed. They were then left in a 13.5 Tesla magnetic field for 62 hours. Diffraction patterns were recorded using a Rigaku RU200 X-ray generator and an R-Axis II imaging plate detector.

Diffraction patterns from some of the best-oriented samples of PMV and PVX are shown in Figure 2. The mean disorientation of the PMV sample (Figure 2a) is about 6°; of PVX (Figure 2b), about 5°.

These diffraction patterns are not sufficiently good for high resolution structure determination, but they are significantly better than any previously obtained. Furthermore, there are excellent possibilities for further improvement. It is clear that the potexviruses respond well to magnetic orientation, and particularly to the combination of centrifugation and magnetic orientation developed by Namba's group. The time of exposure to the magnet in these experiments was much less than optimal [30], and it seems likely that the concentration of the viruses was also less than ideal. Experiments with both viruses are continuing.

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Crystallisation in block copolymer melts: soft-hard templating

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The crystallisation of shear oriented oxyethylene/oxybutylene (E/B) diblock copolymers has been studied by simultaneous SAXS and WAXS. Crystallisation of ordered melts can be accompanied by a change in length scale and retention of the melt orientation. Lamellar melts crystallise with an increase in length scale with multiply-folded E blocks and the B blocks slightly stretched from their melt conformation. Crystallisation from oriented gyroid melts leads to an increase in length scale with preferred melt directions being selected. The retention of layer planes on crystallisation from an ordered melt is caused by the local stretching of chains and the locally one dimensional structure, despite the relative strengths of the structural process. We demonstrate that an interfacial preordering effect can cause crystallographic register to jump length scales in a soft matter system showing epitaxial crystallisation.

Introduction

Self-assembly of amphiphilic molecules provides one of the fundamental structure directing processes for building hierarchical structures in nature¹. The universality of pattern formation by lipid membranes, lyotropic and thermotropic liquid crystals, and block copolymers¹⁻³, all soft-structures