A comparison of the solution structures of tobacco rattle and tobacco mosaic viruses from Raman optical activity

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Vibrational Raman optical activity (ROA) spectra of tobacco rattle virus (TRV) and tobacco mosaic virus (TMV) were measured and compared with a view to obtaining new information about the coat protein subunit structure of TRV. A sharp strong positive band observed at ~1344 cm⁻¹ in the ROA spectra of the two viruses is evidence that both contain a significant amount of a hydrated form of α-helix, but more in TRV than in TMV. Although the ROA spectrum of TMV shows significant positive intensity in the range ~1297–1312 cm⁻¹ characteristic of α-helix in a hydrophobic environment, as expected from the helix interface residues in the four-helix bundles that constitute the basic motif of the TMV coat protein fold, that of TRV shows little positive ROA intensity here. Instead TRV shows a strong positive ROA band at ~1315 cm⁻¹, of much greater intensity than bands shown here by TMV, that is characteristic of polyproline II (PPII) helix. This suggests that the additional long central and C-terminal sequences of the TRV coat proteins contain a significant amount of PPII structure, plus perhaps some β-strand judging by a prominent sharp negative ROA band shown by TRV at ~1236 cm⁻¹, but little α-helix. The open flexible hydrated nature of PPII helical structure is consistent with the earlier suggestions that the additional sequences are exposed and, together with a larger amount of hydrated α-helix, could serve to fill the extra volume required by the larger diameter of the cylindrical TRV particles relative to those of TMV.

Introduction

Tobacco rattle virus (TRV) and tobacco mosaic virus (TMV) belong to the Tobravirus and Tobamovirus genera of plant viruses, respectively. For both genera, genomes of single-stranded positive-sense RNA are encapsidated within rigid helical rod-shaped particles. The detailed structure of TMV has been obtained from X-ray diffraction (Klug, 1999; Stubbs, 1999). However, difficulties with sample preparation have hampered high resolution X-ray diffraction studies on tobraviruses. Direct information on their particle structure has come mainly from electron microscopy (Roberts & Mayo, 1980), but nothing definite is known about the three-dimensional molecular structure of the coat proteins or the RNA, or about the arrangement of the coat protein subunits within the tobravirus particle. The only available model of the protein subunit structure was proposed on the basis of sequence comparisons with tobamoviruses (Goulden et al., 1992).

The coat protein sequences of the tobraviruses contain 196–223 residues, compared with 156–161 for the tobamoviruses. Proteins of both genera possess at their C termini a region of variable sequence, which consists of 15–38 residues in tobraviruses, whereas the corresponding region in tobamoviruses contains only 3–9 residues. Another region of variable sequence, at the N terminus of tobravirus proteins, has no counterpart in tobamoviruses. In addition, tobraviruses have a central insertion of 26–33 residues of variable sequence relative to tobamoviruses. Unlike TMV particles, which give no proton nuclear magnetic resonance (NMR) signals, those of TRV give an NMR signal that has been attributed to segmental mobility of the extra C-terminal sequence (Mayo et al., 1993). Furthermore, the C-terminal region of TRV was found to be strongly antigenic and a central region (residues 110–121) to be more weakly antigenic, suggesting that these regions are exposed externally in the intact virus particle and could be associated with its transmission by nematodes (Legorburu et al., 1996).

The lack of detailed information about the molecular structure of tobraviruses highlights the dearth of physical
techniques generally applicable to the determination of virus structure and function. Of the few techniques available, Raman spectroscopy is particularly useful on account of its potential ability to provide structural information about both protein and nucleic acid constituents of intact virions and viral precursors over a broad range of sampling conditions (Thomas, 1999). A novel form of Raman spectroscopy called Raman optical activity (ROA), which measures small differences in the Raman spectra of chiral molecules acquired using right- and left-circularly polarized incident light, has recently been applied to biomolecules and provides new information on their solution structure and dynamics (Barron et al., 2000). ROA bears the same relation to conventional Raman spectroscopy as does ultraviolet circular dichroism (UVCD) to conventional UV absorption spectroscopy. The largest ROA signals are often associated with vibrational coordinates which sample the most rigid and chiral parts of the structure. In proteins these are usually within the peptide backbone and often give rise to ROA bands characteristic of the backbone conformation, unlike the parent Raman spectra in which many bands from the amino acid side-chains often obscure the peptide backbone bands. As well as bands arising from secondary structure (together with a few bands from side-chains), protein ROA spectra also contain bands from loops and turns and so can provide information about the tertiary fold of the peptide backbone. Nucleic acid ROA spectra contain information on base stacking, the mutual orientations of sugar and base rings, and the sugar-phosphate backbone conformations. The first observations of ROA on intact viruses, specifically filamentous bacteriophages, were published recently (Blanch et al., 1999). Here we report ROA data on the PRN strain of TRV and the U1 strain of TMV from which new information is deduced about the coat protein structure of TRV.

Methods

Particles of TRV strain PRN were purified as described by Robinson (1983) for strain OR. The sample of TMV U1 strain, prepared by the method of Boedeker & Simmons (1958), was kindly supplied by J. J. Milner (University of Glasgow). Both viruses were studied as solutions at ~25 mg/ml in 15 mM phosphate buffer at pH 7.4 held in small quartz microfluorescence cells at ambient temperature (~20 °C). The ROA measurements were performed using an instrument described previously (Hecht et al., 1999). It has a backscattering configuration and employs a single-grating spectograph fitted with a backthinned charge-coupled device (CCD) camera as detector and an edge filter to block the Rayleigh line. The small ROA signals are accumulated by synchronizing the Raman spectral acquisition with an electrooptic modulator that switches the polarization of the incident argon-ion laser beam between right- and left-circular at a suitable rate. The spectra are displayed in analogue-to-digital counter units as a function of the Stokes–Raman wavenumber shift with respect to the exciting laser wavelength. The ROA spectra are presented as circular intensity differences, I<sub>R</sub> − I<sub>L</sub>, and the parent Raman spectra as circular intensity sums, I<sub>R</sub> + I<sub>L</sub>, where I<sub>R</sub> and I<sub>L</sub> are the Raman-scattered intensities in right- and left-circularly polarized incident light, respectively. The experimental conditions were as follows: laser wavelength 514.5 nm; laser power at the sample ~700 mW; spectral resolution ~10 cm<sup>-1</sup>; acquisition time ~48 h.

Results and Discussion

The Raman and backscattered ROA spectra of TRV and TMV are shown as the top and bottom pairs of spectra, respectively, in Fig. 1. An account of most of the ROA assignments given in what follows can be found in Barron et al. (2000). The ROA spectra of the two viruses are dominated by protein bands. No ROA bands from the RNA are apparent, presumably because of the low RNA contents (~5% of the total particle mass in each case). Although useful ROA bands from RNA cover a similar spectral region to those from proteins, the typical band patterns are quite different and so the RNA bands should be readily distinguishable from the protein bands if they were sufficiently intense. It is immediately apparent that the major coat proteins of both viruses contain a significant amount of z-helix, although the actual quantities and characteristics are different between the two. In particular an ROA couplet, negative at ~1361 cm<sup>-1</sup> and positive at ~1664 cm<sup>-1</sup>, originating in peptide amide I modes involving mainly C = O stretching and characteristic of z-helix, is shown by both viruses. The intensity of this couplet in the TMV spectrum is significantly greater than in that of TRV, relative to the other main ROA bands, indicating that the TMV coat proteins contain a greater proportion of z-helix. Positive protein ROA bands in the range ~1297–1312 cm<sup>-1</sup> together with positive bands in the range ~1340–1345 cm<sup>-1</sup>, assigned to extended amide III modes involving peptide backbone N–H and C<sub>α</sub>–H deformations and the C<sub>α</sub>–N stretch, are also characteristic of z-helix. The latter bands have been suggested to arise from a hydrated form of z-helix and the former to z-helix in a more hydrophobic environment. The best evidence for this is provided by the earlier ROA studies on filamentous bacteriophages, since these possess overlapping extended helical sequences containing roughly equal amounts of water-exposed residues and residues at hydrophobic helix–helix interfaces which have been associated with strong positive ROA bands at ~1342 and 1300 cm<sup>-1</sup>, respectively (Blanch et al., 1999). Both TRV and TMV show strong sharp positive ROA bands in the range ~1340–1345 cm<sup>-1</sup> from hydrated z-helix, with that of TRV being stronger relative to other bands than that of TMV. The remarkably sharp narrow appearance of these bands for TRV and TMV suggests that the hydrated helical sequences in both viruses are rather rigid, which implies that the associated water is structured, possibly ice-like (Berghethon, 1998). In contrast, only TMV shows significant positive ROA intensity in the range ~1297–1312 cm<sup>-1</sup> from z-helix in a hydrophobic environment: TRV shows instead a weak shoulder at ~1300 cm<sup>-1</sup> on a strong sharp positive ROA band peaking at ~1315 cm<sup>-1</sup>, characteristic of polyproline II (PPII) helix (side infra). The ROA couplets centred at ~1100 cm<sup>-1</sup>, negative at low wavenumber and positive at
high, shown by both viruses are assigned mainly to C–C, C–N stretching of the peptide backbone and are also often shown by α-helical sequences (but not always, which suggests some dependence on the nature of the side-chains).

The various α-helix ROA bands shown by TMV are therefore consistent with the basic four-helix bundle fold of its coat proteins (Klug, 1999; Stubbs, 1999), which contains both water-exposed residues and residues at hydrophobic helix–helix interfaces (at the time of writing we do not have the ROA spectrum of a model four-helix bundle protein). On the other hand, TRV appears to contain more hydrated α-helix formed at the expense of hydrophobic α-helix. This could still be consistent with a four-helix bundle, but more flexible allowing for more water penetration than in the coat proteins of TMV. For example, although hen lysozyme and equine lysozyme have the same detailed three dimensional X-ray crystal structures containing very similar amounts of α-helix, hen lysozyme shows a strong positive ~ 1300 cm⁻¹ ROA band and a weaker ~ 1344 cm⁻¹ ROA band, whereas the relative intensities of these two bands are reversed for equine lysozyme (Blanch et al., 2000a). This is probably associated with greater flexibility and water penetration of the equine lysozyme structure relative to hen lysozyme, as evidenced by much lower average protection factors from ¹H NMR for amide hydrogen–deuterium exchange in equine lysozyme than in hen lysozyme (Morozova-Roche et al., 1997).

Positive ROA intensity in the range ~ 1315–1325 cm⁻¹ appears to be characteristic of PPII helix, or at least of loop structure containing residues clustering in the PPII region of the Ramachandran surface (Smyth et al., 2001; Blanch et al., 2000b). The positive ROA intensity in this range shown by TMV could therefore arise from some of the loops within the coat protein fold (Klug, 1999; Stubbs, 1999). However, the ROA spectrum of TRV contains much more intensity in this region in the form of a strong sharp positive ROA band at ~ 1315 cm⁻¹, which indicates that the coat protein of this virus contains much more PPII structure than the TMV protein. The prominent negative extended III ROA band of TRV peaking at ~ 1236 cm⁻¹ is characteristic of β-strand, which suggests that TRV contains more of this conformational element than TMV for which the negative ROA intensity in this region is weaker and less well-defined. Since PPII structure is often hydrated, flexible and found mainly in loop regions on protein surfaces (Adzhubei & Sternberg, 1993; Stapley & Creamer, 1999), it is a likely conformational element to be found in the additional C-terminal sequence which the earlier NMR antigenicity work has suggested is exposed externally (Mayo et al., 1993; Legorburu et al., 1996). It therefore seems likely that PPII helix is a significant conformational element in the additional central and C-terminal sequences that are present in the TRV coat protein compared with the TMV protein. There may also be some β-strand in these sequences but little, if any, z-helix.

Goulden et al. (1992) identified amino acid residues, conserved in the coat protein sequences of four tobaviruses, that were counterparts of residues with important functions in the hydrophobic core structure of the TMV coat protein subunit. Inspection of the 13 tobavirus coat protein sequences now available confirms the conserved status of almost all of these residues. Thus, the idea that tobavirus and tocbavivirus coat proteins have similar overall folding patterns, reflecting a common evolutionary origin, and in particular that both structures are based on a four-helix bundle, still seems valid, even though the four-helix bundle of TRV may be more hydrated and open than that of TMV. Mainly due to the larger radius of the cylindrical tobavirus particles, the volume occupied by a tobavirus coat protein subunit is significantly larger than that occupied by a tocbavivirus subunit.

The ROA data suggest that the additional volume of TRV may be associated with large amounts of open hydrated PPII structure together with increased hydration of the α-helical regions. This could be consistent with the structural model of the complete TRV particle proposed by Goulden et al. (1992).
in which corresponding structural elements of the subunits are displaced radially by 4 nm relative to their positions in TMV, the number of subunits per turn is increased from $16\frac{2}{3}$ to $25\frac{2}{3}$, and the subunits acquire an increased bulk. Exposed hydrated PPII structure (plus some \( \beta \)-strand) from the additional central sequence might occupy the vacated volume around the axial hole, that from the additional C-terminal sequences could be present on the outer surface, and additional hydrated \( \alpha \)-helix could provide increased bulk either within the helix bundles by increasing the distances between some helices or externally by increasing the separations between adjacent helix bundles. The present ROA study therefore provides partial experimental support of the model of Goulden et al. (1992), but with some additional details.

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References


