A classification of the tobamoviruses based on comparisons among their 126K proteins

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The products of partial proteolysis of the M, 126000 in vitro translation products of the RNA of eight tobamoviruses were separated by SDS–polyacrylamide gel electrophoresis. The peptide patterns obtained were compared using a computer program designed to establish phylogenetic relationships. The resulting most-parsimonious phylogenetic trees grouped the tobamoviruses into clusters I (tobacco mosaic virus, tomato mosaic virus, tobacco mild green mosaic virus, pepper mild mottle virus) and II (sunn-hemp mosaic virus, cucumber green mottle mosaic virus, kyuri green mottle mosaic virus), with ribgrass mosaic virus in an intermediate position. This clustering resembles that obtained when the coat proteins of these viruses are compared. If the tobamoviruses have arisen by divergence from an ancestral type, the results suggest that different parts of the genome have diverged similarly and that recombination has not played a major role in the evolution of the group.

Introduction

In the absence of data on nucleic acid sequences, studies on taxonomic relationships and differentiation among the members of individual groups of plant viruses have been based largely on comparisons among their coat proteins, using as data amino acid sequences (Gibbs, 1980; Shukla & Ward, 1988), amino acid composition (Fauquet et al., 1986a, b; Gibbs et al., 1982; Paul et al., 1980; Shukla et al., 1988; Tremaine & Argyle, 1970) or serological relatedness (Barnett et al., 1987; Francki et al., 1986; Shukla et al., 1989). However, the coat protein is encoded by a small percentage of the viral genome and the greater part is ignored in these types of comparisons. In addition, variation of the coat protein could be limited by constraints that may not operate on other virus-encoded proteins. Also, recombination may play a role in viral evolution (Allison et al., 1989; Angenent et al., 1989; Bujarski & Kaesberg, 1986; King, 1988; Robinson et al., 1987) and mechanisms of evolution based on the exchange of functional modules have been proposed (Zimmern, 1988). These considerations emphasize the value of comparing other virus-encoded proteins or regions of the viral genome other than the coat protein gene.

The relationships among tobamoviruses have been established either by detailed work on their coat protein (Gibbs, 1986; Van Regenmortel, 1986) or RNA nucleotide sequence homologies as determined by hybridization with homologous and heterologous cDNA (Palukaitis & Symons, 1980; Palukaitis et al., 1981; van de Walle & Siegel, 1982). Although this last method allows a general comparison of the whole of the genomic RNAs, it differentiates viruses within a group rather than providing a quantitative picture of viral relatedness.

The 5′-most cistron of the tobamoviruses encodes a 126K protein that is considered to be part of the viral replicase (Palukaitis & Zaitlin, 1986). The amino acid sequences of the 126K proteins of tobacco mosaic virus (TMV), tomato mosaic virus (ToMV) and tobacco mild green mosaic virus (TMGMV) have been deduced from the sequenced genomic RNAs (Goelet et al., 1982; Ohno et al., 1984; Solis & García-Arenal, 1990). They have 80% (TMV and ToMV) or 65% (TMGMV and ToMV, TMGMV and TMV) identity, but no data are available for other tobamoviruses that would permit a more extensive comparison among members of this group.

Here we report data on the relationships among eight tobamoviruses (TMV, ToMV, TMGMV, pepper mild mottle virus, PMMV, ribgrass mosaic virus, RMV, sunn-hemp mosaic virus, SMMV, cucumber green mottle mosaic virus, CGMMV, kyuri green mottle mosaic virus, KGMMV) based on comparison between the peptide maps obtained by digesting the non-structural 126K protein with different proteases.

Methods

Viruses and RNA preparation. The tobamoviruses used are listed in Table 1. The propagation hosts were Nicotiana tabacum cv. Samsun (TMV, ToMV, TMGMV and RMV), N. clevelandii (PMMV),...
Table 1. Details of the tobamoviruses used

<table>
<thead>
<tr>
<th>Virus</th>
<th>Isolate description</th>
<th>Source</th>
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<tbody>
<tr>
<td>TMV</td>
<td>Tobacco mosaic virus, U1 isolate (Siegel &amp; Wildman, 1954)</td>
<td>M. Zaitlin</td>
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<tr>
<td>TMGMV</td>
<td>Tobacco mild green mosaic virus, U2 isolate (Siegel &amp;</td>
<td>M. Zaitlin</td>
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<tr>
<td></td>
<td>Wildman, 1954)</td>
<td></td>
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<tr>
<td>ToMV</td>
<td>Tomato mosaic virus, Dahlamense strain (Hollings &amp;</td>
<td>M. Zaitlin</td>
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<tr>
<td></td>
<td>Huttinga, 1976)</td>
<td></td>
</tr>
<tr>
<td>SHMV</td>
<td>Sunn-hemp mosaic virus, Cc-TMV strain (Bawden, 1958)</td>
<td>M. Zaitlin</td>
</tr>
<tr>
<td>PMMV</td>
<td>Pepper mild mottle virus, type strain (Wetter &amp; Conti,</td>
<td>M. H. V. Van Regenmortel</td>
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<tr>
<td></td>
<td>1986)</td>
<td></td>
</tr>
<tr>
<td>RMV</td>
<td>Ribgrass mosaic virus, Holmes strain (Oshima &amp; Harrison,</td>
<td>A. Alfaro</td>
</tr>
<tr>
<td></td>
<td>1975)</td>
<td></td>
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<tr>
<td>CGMMV</td>
<td>Cucumber green mottle mosaic virus, CV4 strain (Francki</td>
<td>R. I. B. Francki</td>
</tr>
<tr>
<td></td>
<td>et al., 1986)</td>
<td></td>
</tr>
<tr>
<td>KGMMV</td>
<td>Kyuri green mottle mosaic virus, corresponding to</td>
<td>R. I. B. Francki</td>
</tr>
<tr>
<td></td>
<td>CGMMV-C (cucumber isolate from Japan) (Francki et al.,</td>
<td></td>
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<td></td>
<td>1986)</td>
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</tr>
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</table>

Phaseolus vulgaris (SHMV), Cucumis sativus (CGMMV and KGMMV). Purification was as described by Bruening et al., (1976; TMV, ToMV, TMGMV, RMV and PMMV), or Kassanis & McCarthy (1967; SHMV), or Tung & Knight (1972; CGMMV and KGMMV). RNA was isolated from purified virus particles by phenol extraction and ethanol precipitation (Crum et al., 1988).

126K protein analyses. For each virus, 5 µg of genomic RNA was translated in 20 µl of rabbit reticulocyte lysate (Amersham) containing 30 µCi of [35S]methionine (1489 Ci/mmol) according to the manufacturer's instructions. After translation, samples of 2 µl were taken to assess the amount of protein synthesis by measuring the incorporation of radioactive into trichloroacetic acid-precipitable material (Bruening et al., 1976). Labelled translation products were separated by SDS-PAGE according to Laemmli (1970). Gels were dried and the bands corresponding to the 126K protein were identified by autoradiography and excised. The pieces of gel were treated with proteases and the resulting peptides were separated in an SDS-PAGE according to Cleveland et al. (1977). The protease treatments were with 5 µg per reaction of bovine serum albumin (BSA), and 5 µg of trypsin, chymotrypsin A, Staphylococcus aureus V8 protease (Boehringer Mannheim), elastase type I or thermolysin type X (Sigma). Individual peptides were identified by their positions in the gels and then used as character sets for calculating the relatedness of proteins. Parsimonious unrooted trees (Wagner parsimony, Camin & Sokal, 1965; Kluge & Farris, 1969) were calculated using the Mix program included in the PHYLIP package (version 3.0) of J. Felsenstein (Seattle, Wa., U.S.A.).

Results

The genomic RNAs of TMV, ToMV, TMGMV, PMMV, RMV and SHMV were translated with similar efficiencies in the rabbit reticulocyte cell-free system as judged by the incorporation of radioactivity into acid-precipitable products (approximately 25-fold stimulation over incorporation). KGMMV RNA and CGMMV RNA stimulated incorporation to 11-fold and five-fold, respectively, of the endogenous level. The poor yield of CGMMV 126K protein limited its analysis to treatment with only one protease.

The electrophoretic analysis of the in vitro translation products of the viral RNAs showed a major band in all cases (except for CGMMV), with mobility similar to that of the 126K protein of TMV and a comparatively minor band corresponding to its read-through Mr, 183000 protein (Fig. 1).

The electrophoretic analysis of the partial proteolysis products of the 126K proteins treated with any of the five proteases tested gave a different pattern for each virus (Fig. 2a for V8 and Fig. 2b for thermolysin). The peptides derived from carrier BSA were similar in amount and mobility in each digestion with the same enzyme (Fig. 2a, b; lane 10, for TMV) showing that the extents of the enzyme treatments were comparable. Thus the peptide maps are characteristic of the different 126K proteins analysed and can therefore be used to compare the viruses that encode them.

The complexity of the peptide patterns obtained did not reflect, in general, the specificity of site recognition of the different proteases used. Except for trypsin digestion, the pattern of peptides consisting of 21 to 28 peptides ranging in apparent Mr from 5000 to 30000 (chymotrypsin and protease V8) or from 10000 to 80000 (elastase and thermolysin). With trypsin, the most specific protease assayed, 12 peptides were obtained with M, from 7000 to 57000. The numbers of co-migrating peptides arising from all the 126K proteins were three out of 28 (chymotrypsin), two out of 12 (trypsin), four out of 23 (thermolysin), four out of 21 (V8) and seven out of 28 (elastase).

Comparisons among the 126K proteins

The presence or absence of peptides following treatment with each of the five proteases was used as a qualitative means of calculating the relatedness of the viruses by the
Comparison of 126K proteins of tobamoviruses

Fig. 1. Autoradiography of the in vitro translation products of purified RNAs of different tobamoviruses, separated by 12% SDS-PAGE. Lane 1, TMV; lane 2, TMGMV; lane 3, ToMV; lane 4, PMMV; lane 5, RMV; lane 6, SHMV; lane 7, KGMMV; lane 8, CGMMV. M, markers are shown. The arrows on the right indicate the position of the 126K and 183K proteins.


When the analysis was done with the pooled data obtained with all five proteases, the resulting most-parsimonious unrooted tree grouped the seven assayed tobamoviruses into two main clusters (Fig. 3a): cluster I, including TMGMV, TMV, PMMV and ToMV and cluster II, with SHMV and KGMMV. RMV appears to occupy an intermediate position. When the analysis was done separately with the data generated by each of the five proteases (Fig. 3b and c for V8 and thermolysin, respectively), or after pooling the data from the more specific proteases (trypsin, chymotrypsin and V8) or the less specific ones (elastase and thermolysin), the same two clusters were found.

The trees obtained with these different analyses differ in two respects. Firstly, in the position of RMV which was more or less closely related to cluster I, but always branched before any further divergence within this cluster and secondly, in the relationships among the members of cluster I which varied depending on the enzyme used (Fig. 3b and c). Data for CGMMV were obtained using only thermolysin and this indicated that CGMMV falls into cluster II, more closely to KGMMV than to SHMV (Fig. 3c).

Discussion

We have analysed the relationships among eight tobamoviruses by comparing the peptide patterns resulting from the partial digestion of their 126K proteins with different proteases. As shown by Cleveland et al. (1977) for other proteins, partial proteolysis generated peptides that were characteristic for a particular protein/protease combination. The analysis of this type of data using Wagner’s criterion of maximum parsimony has been widely applied to the study of evolutionary relationships, e.g. among animal viruses (Buonagurio et al., 1986; Dopazo et al., 1988), but has not been used in similar studies with plant viruses, although it appears to be very appropriate to the analysis of data such as those presented here.
The viruses appear to be divided into two clusters (Fig. 3a), cluster I (TMV, ToMV, TMGMV and PMMV) and cluster II (SHMV, KGMMV and CGMMV) with RMV occupying an intermediate position, closer to the members of cluster I. This grouping correlates with some other characteristics; for example, members of cluster II encapsidate the subgenomic mRNA for the coat protein (Higgins et al., 1976; Mesi et al., 1981, 1983; Whitfield & Higgins, 1976) and have restricted natural host ranges (Okada, 1986; Varma, 1986) but not with others, for example KGMMV in cluster II and TMV, ToMV and TMGMV in cluster I are known to be specifically aminoacylated with histidine at their 3' ends (Gibbs, 1986), whereas SHMV is esterified specifically with valine (Beachy et al., 1976).

More notably, the clustering obtained corresponds to that based on sequence homologies (Gibbs, 1980) or amino acid compositions (Gibbs, 1986) of the coat proteins, with minor differences in the relationships among the members of cluster I. It may be that these differences are due to the different sensitivities of comparisons based on sequences rather than peptide maps to detect relationships: although the 126K protein of TMV is more similar to that of TMGMV than that of ToMV (Solis & Garcia-Arenal, 1990), this is not reflected in the number of steps leading from TMV to ToMV or to TMGMV in the trees in Fig. 3. Also the trees based on the digestion of the 126K protein with different proteases do show minor differences in the grouping of members of cluster I, which makes the significance of their relative positions doubtful.

The deduction of very similar patterns of relatedness using the products of the 5' 3400 nucleotides of the 6-4 kb genome (126K protein) and the 3'-most open reading frame (ORF) 480 nucleotides long (coat protein), strongly suggests that if tobamoviruses have diverged from an ancestral type, both ends and presumably all of the genome have diverged at similar rates. Sequence comparisons of the 30K proteins (encoded by about 1000 nucleotides in an internal ORF) of TMV, ToMV, TMGMV, SHMV and KGMMV also support this view (Solis and Garcia-Arenal, 1990). Our data also suggest that no gross recombination events have occurred during the evolution of tobamoviruses. Nevertheless, the possibility of exchange by recombination of smaller parts of the genome, such as that suggested by Mesi et al. (1981) to explain the unusual properties of the 3' end of SHMV, cannot be ruled out. This could explain discrepancies between the ordering of the members of cluster I by comparisons based on their 126K or on their coat proteins.

The phylogenetic model proposed here for the tobamoviruses differs from those which have been described for other plant viruses. The members of the tymovirus group can be ordered differently according to different properties and to sequence data (Blok et al., 1987; Ding et al., 1989, Jellison, 1987), suggesting more complex relationships between them than between the tobamoviruses. The best data from which to attempt to understand evolutionary relationships among members of the tobamoviruses, or any other group, are probably the complete sequences of the genomic RNA of each of the members. In the absence of these data, the method used in this work permits a quick, reliable and easy comparison of different non-structural proteins, which provide a good complement to data based on coat proteins.

Fig. 3. Most-parsimonious Wagner unrooted trees of the compared tobamoviruses. (a) Tree resulting from analysing the data obtained from digesting the 126K protein of seven tobamoviruses with five proteases. (b) Tree resulting from data of the digestion of the 126K protein of seven tobamoviruses with V8 protease. (c) Tree resulting from data of the digestion of the 126K protein of eight tobamoviruses with thermolysin.
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References


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