Sequence Relationships Among the Coat Proteins of Strains of Pea Mosaic, White Lupin Mosaic, and Bean Yellow Mosaic Potyviruses

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ABSTRACT

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The taxonomic relationships of 20 potyvirus isolates from the bean yellow mosaic virus (BYMV) subgroup were investigated by high-performance liquid chromatographic peptide profiling of tryptic digests of their coat proteins. The peptide profiles of the clover yellow vein virus (ClYVV) strains B, C81, LI, and Washington were almost identical to each other and were closely related to that of ClYVV-Pratt, confirming their status as strains of ClYVV and not strains of BYMV. The profiles of BYMV-GDD, G, WA8, WA22, and RL7 were almost superimposable on each other and were similar to the profiles of BYMV-Scott, S, G81, and F, indicating that their coat proteins were very similar. Comparisons of coat protein tryptic peptides from white lupin mosaic virus (WLMV) and three strains of pea mosaic virus (PMV) showed that the peptide profiles of WLMV and PMV-204-1 were very similar to those of BYMV-S, G81, and F, and that peptide compositions closely matched published sequences of BYMV strains. The profiles for PMV-I and Provvidenti were almost identical to that of BYMV-K but showed some differences to the majority of the BYMV profiles. Additional data such as amino acid composition and sequence analysis revealed that these differences were due to just a few amino acid substitutions in their coat protein sequences when compared to those of BYMV-GDD. Many of these substitutions matched those found in the published sequences of BYMV-S, CS, and Danish. The HPLC profiles, together with the amino acid composition and sequence data, indicate that the WLMV and PMV isolates, therefore, are strains of BYMV. Data for the coat protein of sweet pea mosaic virus (SPMV) were more limited and insufficient to establish the relationship of SPMV to the BYMV strains, although several of the sequence changes seen in SPMV were also seen in PMV-I, PMV-Provvidenti, and the published sequence for BYMV-CS. It was also observed that WLMV and the PMV isolates had a conserved C-terminal sequence identical to that of known BYMV sequences but which differed substantially from the conserved C-terminal sequence of the three ClYVV strains. These sequences are candidate epitopes that might assist the assignment of isolates from the BYMV subgroups as strains of BYMV or ClYVV.

Taxonomic relationships among the legume-infecting potyviruses have been difficult to establish because of their overlapping host ranges, symptomatology, and serological interactions. This is particularly true for members of the bean yellow mosaic virus (BYMV) subgroup, which includes BYMV, pea mosaic virus (PMV), sweet pea mosaic virus (SPMV), white lupin mosaic virus (WLMV), and clover yellow vein virus (ClYVV) (2,13,14,29).

The similarities between BYMV, PMV, ClYVV, and SPMV have been concisely summarized by Barnett et al (2), who suggested that ClYVV should be added to the BYMV subgroup of BYMV, PMV, and SPMV that had been proposed by Randles et al (33) on the basis of coat protein amino acid composition. This grouping was based on the findings that the host ranges of these viruses are similar; they induce the formation of the same type of cytoplasmic inclusions (subdivision II); they are the only viruses in subdivision II that induce nuclear inclusion body formation; and they are serologically related to a greater or lesser extent depending on the procedure adopted (2).

While BYMV, ClYVV, PMV, and WLMV are accepted as members of a related subgroup, in some cases it has been disputed whether they are distinct viruses (2,4,10,11,19,33) or strains of the same virus, BYMV (4,18,26,27,36). Molecular hybridization studies with randomly primed cDNA suggested that there was strong sequence identity within strains of each of BYMV, ClYVV, and PMV, but little sequence identity between these three viruses

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or with SPMV, suggesting that they are distinct potyviruses (1,2,34). Similar results were obtained from hybridization studies targeted at the 3′ noncoding regions of ClYVV, BYMV-S, and PMV-I (45). Some of these findings were supported by coat protein and 3′ noncoding gene sequences which showed that BYMV and ClYVV are distinct potyviruses (5,6,12,43–46). However, no sequence data are available for PMV or WLMV. Since ClYVV-30 was originally described as BYMV-30 (43), a strain of BYMV (16,44), it raised the possibility that other viruses in the BYMV subgroup may be incorrectly classified.

Analysis of amino acid and nucleotide sequence data from coat proteins has been shown to be a powerful approach for clarifying the taxonomic status of viruses and strains in the potyvirus group (35,40,41,47,48). Previously we have shown that high-performance liquid chromatographic (HPLC) profiles can be used as indicators of the extent of identity between potyvirus coat proteins, thereby distinguishing strains from distinct viruses (17,22–25). In this study, peptides obtained by tryptic digestion of the coat proteins of 10 strains of BYMV, five strains of ClYVV, three strains of PMV, and one each of WLMV and SPMV were examined. Some of the profiles differed significantly and in some cases more detailed information, such as amino acid composition and sequence data, was of crucial importance in clarifying the relationships between their coat protein sequences.

MATERIALS AND METHODS

Viral strains investigated. Strains of the viruses investigated and their sources are shown in Table 1. The BYMV-F, BYMV-K, and ClYVV-B isolates have only been partially characterized (42) although the coat protein coding and 3′ noncoding region of ClYVV-B has been determined and shown to represent a distinct ClYVV strain (45). WLMV was propagated and purified according to Hampton et al (13). BYMV-F, K, and S; PMV-I; ClYVV-B and SPMV were propogated in broad bean (*Vicia faba*) and purified by the method of Tracy et al (45). All other viruses were propagated in pea (*Pisum sativum*) and purified according to method 2 of Reddick and Barnett (34). Purity was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE; 20).

Preparation of peptides and HPLC profiling. Enzyme digests were prepared by suspending 0.3–0.8 mg of freeze-dried viral preparations in 150–400 μl of 0.05 M ammonium bicarbonate by sonication, followed by incubation overnight at 37 C with trypsin (TPCK-treated, Worthington Biochemical Corporation, Freehold, NJ) at a 1:50 enzyme to protein ratio. Solutions were

TABLE 1. Origin and source of potyvirus isolates studied

Isolate[a]	Origin	Reference
BYMV-GDD	Utah	12
BYMV-G	S.A., Australia	2
BYMV-WA8	W.A., Australia	2
BYMV-WA22	W.A., Australia	2
BYMV-RL7	Vic., Australia	2
BYMV-Scott	USDA type isolate	2
BYMV-S	S.A., Australia	2
BYMV-G81-1	Vic., Australia	2
BYMV-F	Tas., Australia	42
BYMV-K	Tas., Australia	42
WLMV	Idaho	13
PMV-204-1	Kentucky	2
PMV-I	Vic., Australia	2
PMV-Provvidenti	New York	36
ClYVV-C81	Clemson University, SC	34
ClYVV-Washington	Prosser, WA	15
ClYVV-LI	Vic., Australia	2
ClYVV-B	Tas., Australia	42
ClYVV-Pratt	Canada	2
SPMV	S.A., Australia	2

[a] BYMV = bean yellow mosaic virus, ClYVV = clover yellow vein virus, PMV = pea mosaic virus, SPMV = sweet pea mosaic virus, WLMV = white lupin mosaic virus.

dried, vortexed with 250–500 μl of 0.1% trifluoroacetic acid and centrifuged for 2 min at 9,000 *g* in a benchtop centrifuge. Soluble peptides were separated by reverse-phase chromatography as previously described (17,22) using a 5 μ Vydac (The Separations Group, Hesperia, CA) C_{18} column connected to a Perkin Elmer (Norwalk, CT) Series 4 liquid chromatograph, monitoring eluted peaks at 214 nm. The resulting profiles were compared by selecting the 17 tallest peaks from each profile and making pairwise comparisons of their retention times. Peaks observed within the first 4 min, which consisted of injection spikes, unbound fractions, and baseline noise at the commencement of the elution gradient, were omitted from the comparisons. Some fractions were rechromatographed on the C_{18} column at 3 C in order to resolve peptide mixtures.

Amino acid analysis of peptides. Peptide fragments were subjected to vapor-phase hydrolysis at 110 C in 5.8 M HCl containing 0.01% phenol for 20–22 h under N_2 and analyzed on a Water's Amino Acid Analyser (Millipore Corporation, Milford, MA) using an ion-exchange column.

Amino acid sequencing of peptides. Aliquots of 0.2–1.0 nmoles of some peptides were subjected to pulsed liquid sequencing using an Applied Biosystems model 470 sequencer. Blocked N-terminal peptides were deacetylated by trifluoroacetic acid treatment as described by Welner et al (49).

RESULTS

Peptide profiles of the BYMV strains. SDS-PAGE showed that preparations of 20 potyvirus strains belonging to the BYMV subgroup (Table 1) consisted largely of undegraded coat protein (not shown). The HPLC peptide profiles of tryptic digests of these coat proteins are shown in Figure 1. Amino acid compositions of the major peptides from BYMV-GDD, the reference strain used, enabled most of them to be located within the known coat protein sequence (12) as shown in Figure 2.

The profiles of BYMV strains G, WA8, WA22, RL7, Scott, S, G81, and F were very similar to that of GDD. The few differences observed between these BYMV coat protein profiles were the small retardation in the peak **a** of BYMV-G (equivalent to peak 7 in GDD); the appearance of the new peaks labeled **b, c, d, e,** and **h** in G, WA8, WA22, Scott, and G81, respectively; and the variation in mobility of the peaks **f, g,** and **i** that correspond to peak 17 of GDD (Fig. 1).

Amino acid compositions of the peptides within these peaks are shown in Table 2 and were identified by direct sequencing or by comparison with the known coat protein sequences of BYMV-GDD (12), S (45), Danish (5), and CS (43,44). For simplicity the sequences of these accepted BYMV strains (G, WA8, WA22, RL7, Scott, G81, and F) are not included in Figure 2. The peptide in peak **a** of BYMV-G had an identical composition to peptide T7 of GDD (residues 61–65) except for a Ser for Asn substitution at residue 62 (Table 2; Fig. 2). The peptide in peak **b** of BYMV-G had an identical composition (Table 2) to peptide T12 of GDD (residues 34–53; Fig. 2) except for a Leu for Gln substitution at residue 34. Similarly the peptide in peak **h** of G81 was shown by amino acid analysis to also correspond to residues 34–53 of GDD except for four differences; the Leu for Gln change seen in peak **b** as well as the substitution of a Thr, an Ile, and a second Leu for an Ala and two Val residues. The precise locations of these additional changes in this peptide were not established.

The peptide in peak **c** of WA8 was shown by direct sequencing to correspond to residues 18–33 of GDD with four sequence differences, Thr for Asn, Asp for Asn, Asn for Asp, and Gly for Val at residues 22, 24, 26, and 32, respectively. The composition of the peptide in peak **d** from WA22 was similar to that in peak **c** (Table 2) and differed from the composition of the corresponding peptide (residues 18–33) in GDD by only two residues. The composition of the peptide in peak **e** in Scott is also similar to those of peaks **c** and **d** and is assumed to come from the same region of the coat protein (Table 2).

Peak **f,** the last eluting peak in the profile of BYMV-Scott

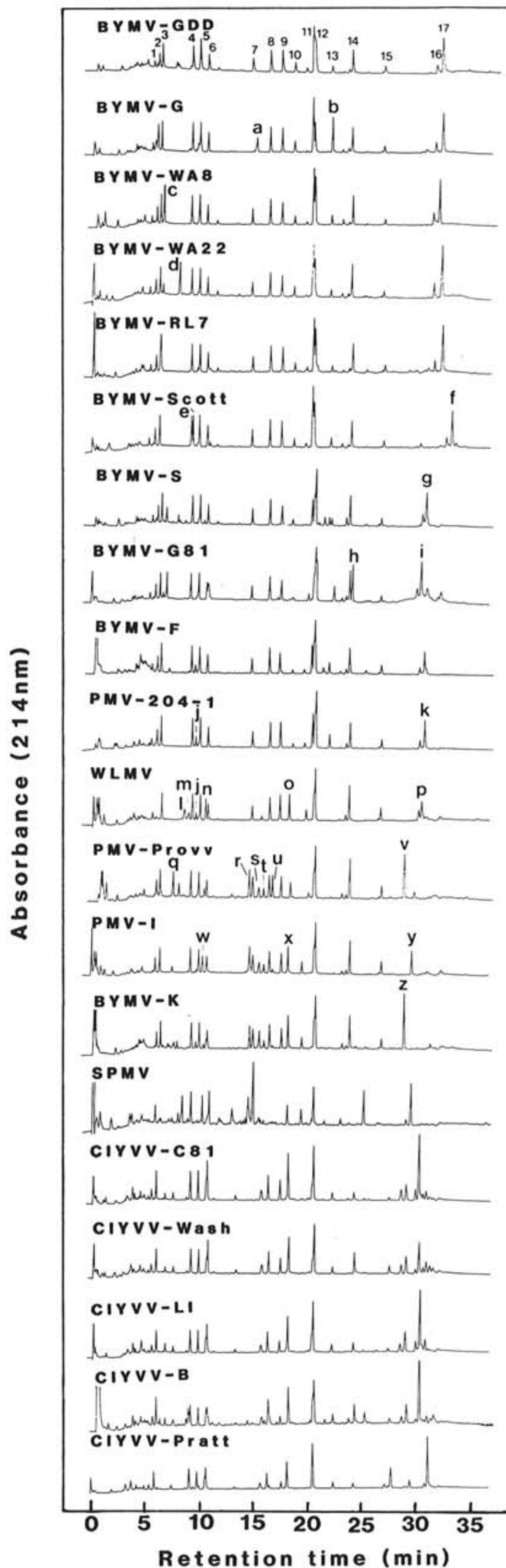

Fig. 1. High-performance liquid chromatographic (HPLC) peptide profiles of tryptic digests of 20 potyvirus isolates belonging to the BYMV subgroup. BYMV = bean yellow mosaic virus; ClYVV = clover yellow vein virus. Wash = Washington strain; PMV = pea mosaic virus. Provv = Provvidenti strain; SPMV = sweet pea mosaic virus; WLMV = white lupin mosaic virus. See Table 1 for sources of these potyviruses. Conditions used for generation of tryptic peptides and HPLC peptide profiles are as described in Materials and Methods. Numbered peaks in the BYMV-GDD profile and their equivalents in other profiles were used for comparisons, together with peaks designated by the letters **a–z**.

(Fig. 1), was found by analysis to contain a peptide which differed by only three amino acids from that in fraction 17 (residues 70–105) of the BYMV-GDD profile (Fig. 2). These differences account for the increased retention time. Differences in elution time of the last major peak (peaks **g** and **i**) in the profiles of BYMV-S, BYMV-G81, and BYMV-F (Fig. 1) were also attributable to a few amino acid differences in the peptide that spans residues 70–105 (Table 2).

The HPLC profile of the tryptic peptides of the coat protein of BYMV-K closely resembled those of PMV-I and PMV-Provvidenti and will be described with them in the next section.

Peptide profiles of BYMV-K and the PMV strains. The peptide profile of PMV-204-1 was very similar to the WLMV and BYMV profiles, especially that of BYMV-F, on which it was nearly superimposable (Fig. 1). All major peaks in the PMV-204-1 profile were subjected to amino acid analysis. The results showed that the peptides within the peaks with identical elution times to peaks 3, 4, 5, 7, 8, 9, 10, 11, 12, 14, and 15 of GDD had identical compositions to that expected from the GDD sequence. Peak 6 in 204-1 had a Gly for Glu substitution at residue 192 as found in the published sequences for CS, Danish, and S (Fig. 2). Peak

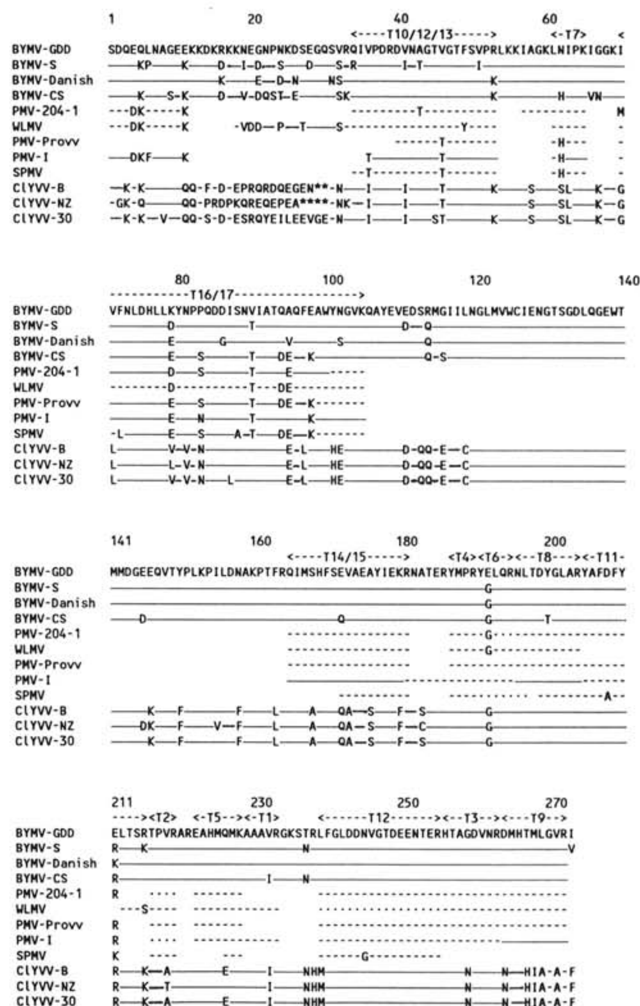

Fig. 2. Alignment of partial amino acid sequences of coat proteins from three pea mosaic virus (PMV) strains, white lupin mosaic virus (WLMV), and sweet pea mosaic virus with sequences of coat proteins from bean yellow mosaic virus (BYMV) strains GDD (12), 5 (45), Danish (5), CS (43,44), clover yellow vein virus (ClYVV) strains B (45), NZ (6), and 30 (46). PMV-Provv = Provvidenti strain. The locations of peptides T1 to T17 from peaks 1 to 17 in the profile of BYMV-GDD (Fig. 1) are shown above the BYMV-GDD sequence. Solid lines indicate sequenced residues identical to those of BYMV-GDD. Dashed lines indicate residues aligned by amino acid compositions of tryptic peptides. Dotted lines show residues aligned on the basis of equivalent retention times of tryptic peptides. Gaps introduced to maximize alignment are shown by *.

j in 204-1 contained the same N-terminal peptide as peak j in WLMV (Table 3; Fig. 2). Peak **k** in the 204-1 profile contained the 36 residue peptide (amino acids 70–105) which by composition (Table 3) and sequence analysis had five differences from the corresponding peptide in GDD, four of which are seen in other BYMV sequences (Fig. 2). These data show that for the peptides compared, the coat protein of PMV-204-1 has 93–97% sequence identity with the coat proteins of known BYMV strains (Fig. 2).

The PMV-Provvidenti and PMV-I profiles were similar to the profile of BYMV-K but differed from the PMV-204-1, WLMV,

TABLE 2. Compositions of variant tryptic peptides from coat proteins of bean yellow mosaic virus (BYMV) strains

Amino acid[d]	G[b] **a**[c]	G **b**	WA8 **c**	WAS22 **d**	Scott **e**	Scott **f**	S **g**	G81 **h**	G81 **i**
	(Residues/molecule)[a]								
Ala	...	1.0	...	...	...	3.9	2.9	...	3.0
Arg	...	2.2	1.1	1.0	2.1	...	...	1.8	...
Asx	...	3.2	3.0	3.8	4.2	5.8	7.6	3.0	7.7
Glx	...	...	4.3	4.2	4.3	5.3	4.0	...	4.1
Gly	...	2.0	2.0	...	1.1	1.2	1.1	2.0	1.0
His	...	...	...	...	...	1.0	1.1	...	1.0
Ile	1.0	0.8	...	...	1.1	2.7	2.6	2.1	1.9
Leu	1.0	1.1	...	...	...	4.0	4.0	2.0	3.9
Lys	1.0	...	2.0	2.0	2.2	1.1	1.0	...	1.0
Met	...	...	...	...	0.8	...	...	...	...
Phe	...	1.0	...	...	...	1.1	1.0	1.1	0.9
Pro	1.0	2.1	1.0	1.0	2.1	2.0	1.9	1.8	1.8
Ser	0.9	0.8	1.9	2.1	1.9	0.9	0.9	1.1	0.9
Thr	...	1.9	1.0	...	...	1.0	1.9	2.9	1.9
Tyr	...	...	...	...	1.0	1.8	1.7	...	1.6
Val	...	3.9	...	1.0	...	2.7	1.7	2.1	2.7
Total[e]	5	20	16	15	20	35	35	20	35
Sequence position[f]	61	34	18	18	18	70	70	34	70

[a] Strain on first line, peak in boldface letters on the second line.
[b] For nomenclature and source of potyviruses, see Table 1.
[c] Peptides were obtained from peaks **a–i** depicted in Figure 1 and correspond to the major peaks with retention times differing from those observed in the majority of the BYMV profiles.
[d] Determined by amino acid analysis of peptide hydrolysates, as described in the text. Cys and Trp were not determined. Values for Ser and Thr were corrected for losses during hydrolysis.
[e] Total number of residues found by analysis.
[f] Peptides were aligned against the BYMV-GDD, S, Danish, and CS sequences in Figure 2. Numbers refer to the sequence position of the first residue in the aligned peptide. For peaks **c** (WA8) and **g** (BYMV-S), the alignment was confirmed by sequence analysis.

TABLE 3. Compositions of variant tryptic peptides from coat proteins of white lupin mosaic virus (WLMV), bean yellow mosaic virus (BYMV-K) and the pea mosaic virus (PMV) strains[a]

Amino acid[c]	WLMV **j**[b]	WLMV **l**	WLMV **m**	WLMV **n**	WLMV **o**	WLMV **p**	PMV-204-1 **j**	PMV-204-1 **k**	PMV-Provvidenti **q**	PMV-Provvidenti **r**	PMV-Provvidenti **s**	PMV-Provvidenti **t**	PMV-Provvidenti **u**	PMV-Provvidenti **v**	PMV-I **w**	PMV-I **x**	PMV-I **y**	BYMV-K **z**
	(Residues/molecule)																	
Ala	1.0	...	...	...	1.0	2.0	1.0	2.0	3.0	1.1	1.1	1.1	1.0	1.0	1.0	1.0	2.2	1.2
Arg	...	1.1	1.1	1.1	2.1	...	...	...	2.1	...	...	...	1.1	...	...	2.2	...	...
Asx	3.0	4.0	4.2	...	3.0	9.1	3.0	8.3	2.9	1.1	1.1	1.1	2.0	7.3	3.0	3.1	6.6	7.2
Glx	2.2	1.9	2.5	...	1.1	4.3	2.2	5.5	4.3	...	...	...	...	4.4	2.2	1.3	4.5	4.3
Gly	1.0	0.9	1.1	...	2.0	1.1	1.0	1.1	...	1.1	1.1	1.1	2.0	...	1.0	1.9	...	...
His	...	...	...	...	...	1.0	...	1.0	...	...	...	...	...	1.1	...	...	1.3	1.0
Ile	...	...	...	...	0.5	2.4	...	2.0	1.8	...	...	...	...	2.7	...	1.0	2.7	3.0
Leu	1.0	...	...	1.0	...	4.1	1.0	4.2	...	...	...	...	...	4.3	...	...	4.1	4.2
Lys	2.0	1.0	2.1	...	...	0.8	1.9	0.9	1.1	1.0	1.0	1.0	...	1.0	1.9	...	0.7	1.1
Met	...	...	...	...	...	...	...	0.8	...	...	...	...	...	...	...	...	...	...
Phe	...	...	...	...	...	1.0	...	1.0	...	...	...	...	0.9	1.0	0.8	1.0	1.1	1.0
Pro	...	1.9	1.9	1.0	2.0	2.0	...	1.0	0.9	...	...	...	1.0	1.0	...	2.1	1.2	1.0
Ser	0.9	2.0	1.7	1.9	0.9	0.9	0.8	2.0	...	...	...	...	1.0	1.6	0.8	1.0	1.4	1.8
Thr	...	1.0	0.9	2.0	1.8	1.8	...	1.9	...	...	...	...	2.9	1.8	...	3.9	2.0	2.0
Tyr	...	...	...	...	0.8	1.9	...	1.8	...	0.8	1.0	1.0	...	1.0	...	...	1.0	1.0
Val	...	1.1	1.0	0.9	3.4	1.4	...	1.8	...	1.0	1.0	1.0	1.9	0.7	...	2.0	0.8	1.0
Total[d]	11	15	16	8	20	35	11	35	16	6	6	6	14	29	11	20	29	29
Sequence position[e]	1	19	18	212	34	70	1	70	19	99	99	99	40	70	1	34	70	70

[a] For nomenclature and source of potyviruses, see Table 1.
[b] Peptides were obtained from peaks **j–z** depicted in Figure 1 and correspond to the major peaks with retention times differing from those observed in most of the BYMV profiles.
[c] Determined by amino acid analysis of peptide hydrolysates, as described in text. Cys and Trp were not determined. Values for Ser and Thr were corrected for losses during hydrolysis.
[d] Total number of residues found by analysis.
[e] Peptides were aligned against the BYMV-GDD, S, Danish, and CS sequences in Figure 2. Numbers refer to the sequence position of the first residue in the aligned peptide. For peptides **k, l, r, u, v, w,** and **y** the alignment was confirmed by sequence analysis.

and other BYMV profiles by the presence of single (**v**, **y**, and **z**, respectively) rather than multiple (T16, T17) peaks at the end of the profile, and by the appearance of multiple new peaks (**r**, **s**, and **t**) eluting between 15 and 18 min (Fig. 1). Amino acid composition and sequence data were therefore required to establish the molecular basis of these more marked profile differences and to ascertain whether these three isolates were also strains of BYMV, or strains of a distinct potyvirus, pea mosaic virus. The data (Table 3 and Fig. 2) revealed that several of the profile differences were due to the single substitution of Lys for Glu at residue 98 in PMV-Provvidenti and PMV-I (and presumably BYMV-K). This substitution resulted in additional tryptic cleavage of peptide 70–105 into two fragments, 70–98, and 99–105. The larger fragment (residues 70–98) eluted as a single peak in each profile (**v**, **y**, and **z** for PMV-Provvidenti, PMV-I, and BYMV-K, respectively), whereas the smaller fragment (residues 99–105) eluted as multiple peaks (**r**, **s**, and **t**), due to partial photodegradation of the tryptophan residue at position 100 into several products (23). In this respect, it is interesting to note that peaks 16 and 17 in the BYMV-GDD profile had the same apparent amino acid composition (tryptophan is destroyed during acid hydrolysis), corresponding to residues 70–105, indicating that one was a photodegradation product of the other. It is probable that in each of the first 11 profiles (Fig. 1), the final pair of peaks equivalent to BYMV-GDD peaks 16 and 17 contains photodegraded and undegraded forms of the equivalent peptide.

Other peaks that differed in the profiles of PMV-Provvidenti, PMV-I, and BYMV-K were also examined. Amino acid comthe peptide equivalent to peak **u** in the PMV-Providdenti profile was identical to residues 40–53 in BYMV-GDD except for a Thr for Val substitution at residue 46; 2) the peptide in fraction **w** in PMV-I corresponded to the first 11 residues of the coat protein and differed from GDD at four positions; 3) the peptide in peak **x** in PMV-I was identical to 34–53 in BYMV-GDD except for Thr to Val substitutions at positions 36 and 46; 4) the peptide equivalent to peak 7 of GDD, between peaks **r** and **s** in the profiles of PMV-Provvidenti, PMV-I, and BYMV-K (Fig. 1), was identical to residues 61–64 in BYMV-GDD except for a His for Asn substitution at residue 62; and 5) the peptide in fraction **z** of BYMV-K was identical in composition to the sequenced peptide **v** from PMV-Provvidenti. Finally, the PMV-I, PMV-Provvidenti, and BYMV-K peptides with retention times equivalent to peptides T1–T6, T8, T9, T11, T12, T14, and T15 from BYMV-GDD were shown to have the same compositions and/or sequence as their BYMV-GDD counterparts (Fig. 2). Based on the similarity of the peptide profiles obtained, the amino acid substitutions found in the PMV-I and PMV-Provvidenti peptides were assumed to be present in the corresponding peptides of BYMV-K.

Peptide profiles of WLMV. The WLMV profile was very similar to those of BYMV-F and PMV-204-1, differing only in a few peaks (Fig. 1). Amino acid analysis showed that the composition of the WLMV peptides equivalent to those in peaks 1, 3, 5, 7, 8, 9, 14, and 15 of GDD matched the known sequences of the BYMV-GDD coat protein. This is summarized in Figure 2. The new peak **j** in the WLMV profile, contained a blocked peptide with a composition (Table 3) that matched the N-terminal 11 residues of the coat protein of PMV-I except for the Phe for Leu substitution at residue 6, and differed from the GDD sequence in three positions (Fig. 2). The peptide in the new peak **l** was analyzed and sequenced and shown to correspond to residues 19–33 (Fig. 2). Peak **m** had an identical composition to that of **l** except for an additional lysine (Table 3), reflecting partial tryptic cleavage between residues 18 and 19 (Fig. 2). Two peaks with retention times unique to WLMV (peak **n**, residues 212–219; peak **o**, residues 34–53), in each case differed by a single amino acid from the composition of the equivalent peptide within the BYMV-GDD sequence (Fig. 2). Finally peak **p** in the WLMV profile contained a peptide corresponding to residues 70–105 with a composition (Table 3) consistent with only four sequence changes as shown in Figure 2. Together the data indicated that the WLMV coat protein shared 92–96% identity with the BYMV coat protein sequences across the identified regions.

Peptide profile of SPMV. In contrast to the relative similarity of the BYMV, PMV, and WLMV peptide profiles, that of SPMV contained several differences. Fewer than half the peaks shared retention times with those of the other profiles (Fig. 1). Limited amino acid composition and sequence analysis, however, showed that these SPMV peptides corresponded to the peptides in peaks 2, 4, 5, 7, 8, 10, 11, 12, 14, 15, 16, and 17 of GDD. These data are summarized in Figure 2 and show that of the 13 sequence differences detected, 10 were present in the corresponding peptides of the other BYMV/PMV strains examined (Fig. 2). The recovery of new smaller peptides suggests additional changes to Lys or Arg have occurred at residues 171, 198, and 225 in SPMV (Fig. 2).

Peptide profiles of ClYVV strains. The HPLC peptide profiles of five strains of ClYVV are also shown in Figure 1. No substantial differences were observed between the peptide profiles of ClYVV strains C81, Washington, LI, and B (Fig. 1). The peptide profile of ClYVV-Pratt, previously recognized as an aberrant ClYVV strain (2), differed from the other ClYVV strains in having altered retention times for the late-eluting peaks, but its overall peptide profile was clearly that of a ClYVV strain. The multiple peaks around 30 min in the ClYVV profiles (Fig. 1) are presumably caused by photodegradation products of the tryptophan at position 100 in the peptide corresponding to residues 67–105 (Fig. 2) as found for the BYMV profiles.

DISCUSSION

Of the many parameters available to establish the taxonomic status of potyvirus isolates, coat protein and gene sequence data have been shown to be most useful and have been successfully applied to discriminate between potyviruses and strains (35,40,41,47,48). Sequences of the coat protein coding region of the BYMV strains GDD, CS, D, and S and the ClYVV strains B, 30, and NZ (5,6,12,43–46) showed that they were strains of two distinct viruses, and these sequences have been used as references for the present analysis.

Since it is not practical to determine the sequences of a large range of viral coat proteins, simpler techniques such as N-terminal serology (37), cDNA hybridization (2,45), or HPLC peptide profiling (39) have been used to facilitate structural comparisons. N-terminal serology did not remove the strong serological cross-reactions between BYMV and ClYVV (9), indicating the existence of restricted common epitopes within the coat protein sequences that cause an unexpected paired relationship (41), thereby negating this approach as a means of discriminating between these distinct potyviruses in the BYMV subgroup. There are other examples of unexpected paired relationships that highlight the limitations of N-terminal serology in potyvirus taxonomy (38).

Using random cDNA probes to the whole genome, molecular hybridization (2) has shown that six ClYVV isolates (LI, Q1, RL1, NSW, P3, and PQ) from Australia were closely related to each other and more distantly to the U.S. isolate ClYVV-Pratt. In addition the Australian isolates BYMV-Q, G, S, G81, RL7, WA8, and WA22 were confirmed as strains of BYMV and showed strong identity to BYMV-Scott (with BYMV-S the most distantly related). These studies also indicated that PMV-204-1 was not closely related to the well-characterized PMV-I or to any of the BYMV, ClYVV, or SPMV isolates tested (2).

The results in the present paper support the molecular hybridization findings (2) with the ClYVV isolates, but extend the conclusions regarding the other viruses. The HPLC profiles clearly show that the coat protein sequences of ClYVV-C81, ClYVV-LI, and ClYVV-Washington are very similar to each other and to ClYVV-B (whose sequence is known; 45), and that ClYVV-Pratt, while more distant is nevertheless recognizable as a strain of ClYVV (Fig. 1). The data also indicate that none of the BYMV or PMV isolates examined are strains of ClYVV.

The HPLC peptide profiles of the BYMV, PMV, WLMV, and SPMV strains are more difficult to interpret than are those of the ClYVV strains and provide an example of the problems that may be encountered in using peptide profiles to determine coat protein relationships. In previous studies, profiles that were essen-

tially similar to each other have been interpreted as indicating close sequence identity among potyvirus coat proteins (17,22–25). To date, this interpretation has been supported by more detailed analyses. However, determining the correct relationship between sequences from peptide profiles that have a number of differences, as are found here, is not as straightforward. These differences may result from quite distantly related sequences, or may simply be due to a few changes that nevertheless have substantial effects on peptide cleavage and retention patterns. Further data such as amino acid composition and sequence analyses are therefore required to distinguish these possibilities.

Such analyses showed that the differences between the peptide profiles of PMV-Provvidenti, PMV-I, and BYMV-K on the one hand, and BYMV-GDD, G, WA22, WA8, RL7, Scott, S, and F on the other, could be explained in terms of a few residue substitutions, most of which appeared to be common to PMV-Provvidenti, PMV-I, and BYMV-K (Fig. 2). The degree of variability observed in the regions of the coat proteins studied here is the same as that seen in sequenced strains of BYMV (Fig. 2) and less than the level of variation seen in BYMV/ClYVV comparisons. The coat proteins of WLMV, PMV strains 204-1, I, and Provvidenti and BYMV-K appear to be as closely related to those of other known BYMV strains as the latter are to each other. From these analyses it is concluded that PMV-Provvidenti, PMV-I, PMV-204-1, and WLMV could be considered strains of BYMV.

Much of the published literature supports this conclusion. PMV was originally considered to be a virus distinct from BYMV because of its bright yellow mosaic versus mild mosaic symptoms in pea and broad bean and its inability to infect *Phaseolus vulgaris* (3). However, many investigators now consider BYMV and PMV to be strains of the same virus, based on 1) similarities of symptom expression and physical properties (11); 2) the capacity of PMV to infect some strains of *P. vulgaris* (4); and 3) the presence of strong serological cross-reactions with differences that are within the range seen between known strains of BYMV (4). Bos (3) classified PMV as a strain of BYMV and Edwardson and Christie (9) refer to PMV as the pea mosaic strain of BYMV. Against this view is data which shows that resistance to BYMV (7) and PMV (30) is controlled by separate genes in *Pisum sativum*. However, since the molecular basis of host resistance is not known (21), it is difficult to determine how much weight should be given to this characteristic when assessing taxonomic relationships.

In a recent publication it was concluded that WLMV was a distinct virus rather than a strain of BYMV (13) from a consideration of the differences in host-range, symptomatology, serology, and peptide profiles. However, close analysis of these data reveals that the observed differences are within the ranges seen between accepted strains of other potyviruses (9,22,28,32). The peptide profile differences were similar to those found here but were not quantitated by additional amino acid composition and sequence data which would have revealed that only a few residue differences in the coat proteins were responsible for the profile differences between WLMV and the BYMV profiles. While the pattern of reactivities of WLMV to a panel of 22 monoclonal antibodies was in some respects unique, WLMV was found to be similar to other BYMV strains. It contained two of three BYMV-subgroup specific epitopes, five of seven BYMV-specific epitopes, and one highly specific epitope previously found only in BYMV-GDD (13). In addition, the host range and symptomatological data (13) may not be sufficiently different to justify the conclusion that WLMV represents a distinct potyvirus. An example of potyvirus strains showing substantial variation in biological properties is given by the strains of papaya ringspot virus, PRSV-P and PRSV-W, which show substantial host range and symptom variation (31), with only PRSV-P being able to infect papaya (9). Pathotype differences should be regarded as strain discriminators, not species discriminators, since it is known that host range can be expanded by successive passage through intermediate host plants.

The previous inability to detect a close relationship between BYMV, PMV, and SPMV by cDNA hybridization (2,45) may be due to limitations of the hybridization technique. For example no hybridization was observed between PMV-I and PMV-204-1 (2), or between BYMV-S and PMV-I (45), and that between some BYMV strains (S and Scott) was weak (2).

The relationship of SPMV to other members of the BYMV subgroup cannot be established by the present studies. Although the peptide profile of SPMV is distinct from those of the BYMV and CYVV isolates, the limited coat protein sequence data shown in Figure 2 reveal that most of the sequence changes seen in SPMV are also found in the BYMV strains. However at this stage there are insufficient data to conclude whether SPMV should be considered a strain of BYMV or a separate potyvirus. Complete sequence data for SPMV may resolve this matter, in particular, knowledge of the sequence of the C-terminal residues (this peptide from SPMV was not recovered), since our studies have shown that residues 239–272 are conserved across the seven BYMV, PMV, and WLMV sequences (Fig. 2). It is interesting to note that within this same region there is complete conservation of residues among the three ClYVV sequences. Six of the nine terminal residues in ClYVV differ from those in BYMV. These observations suggest that antibodies raised against the C-terminal regions of BYMV and ClYVV could form the basis of a serological method for distinguishing potyvirus isolates belonging to the BYMV subgroup.

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