Confirmation that the Sugarcane Mosaic Virus Subgroup Consists of Four Distinct Potyviruses by Using Peptide Profiles of Coat Proteins

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ABSTRACT

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The taxonomic status of sugarcane mosaic virus (SCMV) strains reported from various parts of the world has been uncertain. Previous analyses of 17 SCMV strains with virus-specific polyclonal antibodies demonstrated that they represent four distinct potyviruses, namely Johnsongrass mosaic virus (JGMV), maize dwarf mosaic virus (MDMV), sorghum mosaic virus (SrMV), and SCMV. To confirm this classification, we have now compared six strains of SCMV, three strains of JGMV, and two strains each of MDMV and SrMV using high-performance liquid chromatographic (HPLC) peptide profiling of tryptic digests of their coat proteins. The results showed that peptide profiles could be divided into four distinct groups, which are as different from each other as are peptide profiles of other distinct potyviruses. These findings confirm our previous classification of SCMV strains based on N-terminal serology.

Sugarcane mosaic virus (SCMV) is a definitive member of the potyvirus group (7). It causes serious disease in sugarcane, maize, and sorghum worldwide (10,24). On the basis of biological properties, a large number of strains of this virus have been reported from different parts of the world (1,10,17,24). Until recently these strains were believed to belong to the same virus, since the majority induced similar symptoms in sugarcane, maize, and sorghum, and they appeared to be interrelated serologically (10,24).

Recent immunochemical studies have shown that antibodies directed to the surface-exposed N-terminus of potyvirus coat proteins are virus specific and react with most strains of individual viruses, whereas those directed to the conserved core region also recognize other distinct potyviruses (16,19). On the basis of this information, Shukla et al (13) developed a simple chromatographic procedure to isolate virus-specific antibodies from antisera to intact potyvirus particles. Reaction of these virus-specific antibodies with 17 SCMV strains from Australia and the United States demonstrated that they could be divided into four distinct potyviruses, namely maize dwarf mosaic virus (MDMV), Johnsongrass mosaic virus (JGMV), sorghum mosaic virus (SrMV), and SCMV (18). A limitation of this technique is that it cannot differentiate between cross reactions that reflect the extensive sequence homology found between strains, and cross reactions termed unexpected paired relationships (13,16,21,22). The latter have been observed in serological tests between some potyvirus isolates that on biological and other grounds have been considered to be distinct viruses (13,16,21,22) and are caused by the presence of short sections of homologous sequences in otherwise unrelated N-terminal regions of coat proteins (22).

Structural properties of the coat proteins have been used to clearly discriminate between distinct viruses and strains in the potyvirus group (20). In that study, distinct members of the group were shown to possess sequence homology of 38–71%, with major differences observed in the length and sequence of their N-termini, whereas strains of individual viruses exhibited sequence homology of greater than 90% and had N-terminal sequences that were very similar (20). Although the determination of protein or gene sequences of every suspected strain of a potyvirus would allow appropriate classification in nearly all cases, the experimental enormity of this task renders it impractical. A simpler alternative is to compare peptide profiles of coat protein digests, since it has been shown that peptide profiles of strains of individual potyviruses are frequently similar, whereas those from distinct viruses are substantially different (14). The procedure reflects the extent of amino acid sequence identity between proteins and gives data based on a comparison of most of the coat protein sequence, unlike serological methods, which focus only on those regions

TABLE 1. Sources of potyviruses used in this study

Virus[a]	Source
JGMV-JG	D. D. Shukla (18)
JGMV-MDMV-KS1	S. G. Jensen (4)
JGMV-MDMV-O	S. G. Jensen (4)
MDMV-A (type)	S. G. Jensen (4)
MDMV-A (Texas)	R. W. Toler (3)
SCMV-MDMV-B	R. W. Toler (3)
SCMV-A	S. G. Jensen (4)
SCMV-D	G. T. Benda (18)
SCMV-SC	D. D. Shukla (18)
SCMV-BC	D. D. Shukla (18)
SCMV-Isis	D. S. Teakle (23)
SrMV-SCMV-H	R. W. Toler (3)
SrMV-SCMV-M	S. G. Jensen (4)

[a]JGMV = Johnsongrass mosaic virus; MDMV = maize dwarf mosaic virus; SCMV = sugarcane mosaic virus; SrMV = sorghum mosaic virus.

of sequence that are presented as major epitopes (possibly only a few amino acids within the N- and C-terminal regions of the molecule—see 19). Peptide profiling of coat proteins recently was used successfully to establish that MDMV-KS1 and MDMV-O are strains of JGMV (9).

In this paper we have compared the tryptic peptide profiles of coat protein digests from six strains of SCMV, three strains of JGMV, and two strains each of MDMV and SrMV. The results confirm the recent classification of the SCMV potyvirus subgroup using N-terminal serology (18) and show that it reflects the sequence relationship of coat proteins of these strains, rather than unpaired serological relationships.

MATERIALS AND METHODS

Virus strains and their purification. The strains of the four viruses investigated are shown in Table 1 along with their sources. JGMV-JG, SCMV-SC, SCMV-BC, SCMV-Isis, and MDMV-D were purified according to the method of Shukla et al (13); MDMV-A (type), SCMV-A, and SrMV (SCMV-M) according to Jensen et al (4); MDMV-A (Texas), MDMV-B, and SrMV (SCMV-H) according to Giorda et al (3). The strains were propagated in either maize (cultivars Gold Cup and Iochief) or sorghum (cultivars RTx 7078 and BTx 623).

Isolation of coat protein. Coat protein from the strains was isolated by disrupting virus in 60% formic acid and pelleting nucleic acid material by centrifugation, followed by dialysis and lyophilization of the coat protein (13). In some cases, prolonged storage at 4 C resulted in coat protein that was seriously degraded. In these cases, preparative polyacrylamide gel electrophoresis was used to obtain samples enriched in undegraded coat protein, using an Applied Biosystems (Foster City, CA) model 230A high-performance electrophoretic chromatography (HPEC) instrument.

Tryptic digestion of viral coat protein. Freeze-dried coat protein was suspended in 0.05 M NH_4HCO_3 (500 μl/mg of coat protein), sonicated 4–5 s to dissolve protein and trypsin treated with tosyl-L-phenylalanine chloromethyl ketone (Worthington Biochemicals, Freehold, NJ), and added as a freshly made solution in 0.05 M NH_4HCO_3 (1,000 μl per 100 μg enzyme) at an enzyme/protein ratio of 1:50. Solutions were kept at 37 C for 16–17 h, dried under vacuum in a Savant Speed Vac Concentrator model 200 H (Hicksville, NY), and redissolved in 0.1% trifluoroacetic acid (TFA—500 μl/mg of coat protein). The solutions were vortexed, and insoluble material was removed by centrifugation for 2 min at 13,000 rpm in an MSE microcentrifuge (Crawley, Sussex, UK).

HPLC profiles of tryptic peptides. Aliquots of soluble tryptic peptides from coat protein digests were loaded onto a Vydac 5 μm C_{18} reverse-phase column (4.6 × 250 mm—Vydac Corp., Hesperia, CA), connected to a Perkin-Elmer Series 4 liquid chromatograph (Norwalk, CT). Components were separated in 0.1% TFA at a flow rate of 1 ml/min using a linear gradient of 0–35% acetonitrile over 60 min at 45 C. A Pharmacia (Uppsala, Sweden) UV-M monitor (214 nm) was used to detect peptides eluting from the column, which was purged at the end of each run by applying a gradient of 35–70% acetonitrile in 0.1% TFA over 2 min, followed by reequilibration in 0.1% TFA for an additional 8 min.

RESULTS

A comparison of HPLC peptide profiles of tryptic digests of coat protein from 13 potyvirus strains, all of which belong to the SCMV subgroup, revealed that they could be divided into four distinct patterns. The peptide profiles of JGMV-JG, MDMV-KS1, and MDMV-O were very similar (data not shown), as demonstrated previously (9). Pairwise comparisons of the profiles showed that 76–86% of peaks had similar retention times (Table 2). HPLC profiles of tryptic peptides from coat proteins of SCMV-A, SCMV-D, SCMV-Isis, SCMV-SC, SCMV-BC, and MDMV-B showed substantial similarity to each other (Fig. 1). Pairwise comparisons of the profiles showed that between 70 and 95% of the peaks had similar retention times (Table 2). Close inspection of the data suggested the presence of two subsets within this set of viruses. The HPLC peptide profiles of SCMV-A, SCMV-D, SCMV-Isis, and SCMV-SC had a greater percentage of peaks with common retention times than those of SCMV-BC and MDMV-B, which themselves showed close similarity. Comparison of HPLC profiles of two isolates of the A strain of MDMV (Fig. 2) revealed that 79% of peaks had similar retention times, substantially greater than their similarity to the other coat protein profiles (Table 2). An analogous situation was observed when HPLC peptide profiles of SCMV-H and SCMV-M were compared (Fig. 3). It was found that 85% of peaks had common retention times (Table 2), whereas pairwise comparison with the other profiles showed that only 19–50% of peaks had common retention times. When a comparison was made of HPLC peptide profiles of one coat protein from each of the four groups defined by Shukla et al (19), four distinct patterns were observed (Fig. 4).

DISCUSSION

The validity of using HPLC peptide profiles of coat protein as a means of assessing the relatedness of potyvirus isolates rests

TABLE 2. Comparison of retention times of peaks from high-performance liquid chromatography profiles of potyvirus coat proteins[a]

	Number of peaks (%) with similar retention times[b,c]												
Potyvirus	JGMV-JG	MDMV-O	MDMV-KS1	SCMV-A	SCMV-D	SCMV-Isis	SCMV-SC	SCMV-BC	MDMV-B	MDMV-A (type)	MDMV-A (Texas)	SCMV-H	SCMV-M
JGMV-JG	100												
MDMV-O	76	100											
MDMV-KS1	81	86	100										
SCMC-A	24	29	24	100									
SCMV-D	33	24	33	87	100								
SCMV-Isis	19	29	29	87	91	100							
SCMV-SC	29	33	33	83	86	87	100						
SCMV-BC	24	19	29	74	70	70	71	100					
MDMV-B	24	24	24	78	70	77	71	95	100				
MDMV-A (type)	19	24	24	25	35	37	42	42	37	100			
MDMV-A (Texas)	29	24	29	37	37	40	37	32	32	79	100		
SCMV-H	24	24	19	48	50	50	44	50	50	50	50	100	
SCMV-M	19	24	19	48	43	50	50	39	48	45	45	85	100

[a] Solid lines enclose comparisons in which greater than 50% of peaks had similar retention times.
[b] Peaks with retention times within 0.3 min of each other were considered to be similar.
[c] The first sample-injection peak and the last column-cleaning peak, common to all profiles, were omitted from comparisons.

on two assumptions. The first is that similarity of peptide profiles reflects a substantial degree of amino acid sequence identity of the coat proteins being used. The second assumption is that the degree of identity of coat proteins at the amino acid sequence level appropriately reflects the taxonomic relationship of the potyvirus isolates.

Previous studies have shown (14) that HPLC peptide profiles of coat proteins of distinct potyviruses were substantially different and bore little or no obvious relationship in their patterns. On the other hand, peptide profiles of coat proteins from known strains of a potyvirus have been found to be quite similar, the majority of peaks having the same retention time and relative peak height. An example of the latter is the similarity of coat protein peptide profiles from three strains of passion fruit woodiness virus (PWV). Biological and serological properties of PWV-Tip blight, PWV-mild, and PWV-severe indicate that they are strains of the one potyvirus, and amino acid sequencing studies have shown that their coat proteins have greater than 95% identity (15).

A second example of strains of a potyvirus having similar peptide profiles comes from the studies of Shukla et al (12), who used similarity of peptide profiles as one of the criteria to establish that the SCMV isolates SCMV-SC, SCMV-BC, and SCMV-Sabi were strains of the one potyvirus, whereas JGMV-JG, also thought to be a strain of SCMV, was shown to be a quite distinct potyvirus. Sequence determination of the coat proteins of these four potyvirus isolates showed that the degree of similarity of peptide profiles was a reflection of their relatedness at the amino acid sequence level (12).

More recently, McKern et al (9) showed that the peptide profiles of JGMV-JG, MDMV-O, and MDMV-KS1 were very similar. On the basis of this similarity, assisted by amino acid analysis of a small number of peptides, it was predicted that the sequences of these coat proteins would differ by not more than 20 of the 303 known amino acid residues (93% identity) of JGMV-JG. Subsequent sequence studies of coat protein of MDMV-KS1 and MDMV-O have confirmed this prediction (5).

To enable a quantitative assessment to be made of the degree of similarity that might exist between HPLC peptide profiles of coat proteins of strains of one potyvirus, the known strains described above were examined, together with profiles of four strains of potato virus Y (14). As a first approximation, retention times (but not peak heights) of elution peaks were compared, not including unbound peaks eluting within the first 3–4 min. An average of 74 ± 13% of peaks (range 57–100%) were found to have identical retention times in 15 pairwise comparisons of peptide profiles of coat proteins from 13 potyvirus strains (data not shown).

In contrast to this high degree of identity of retention times

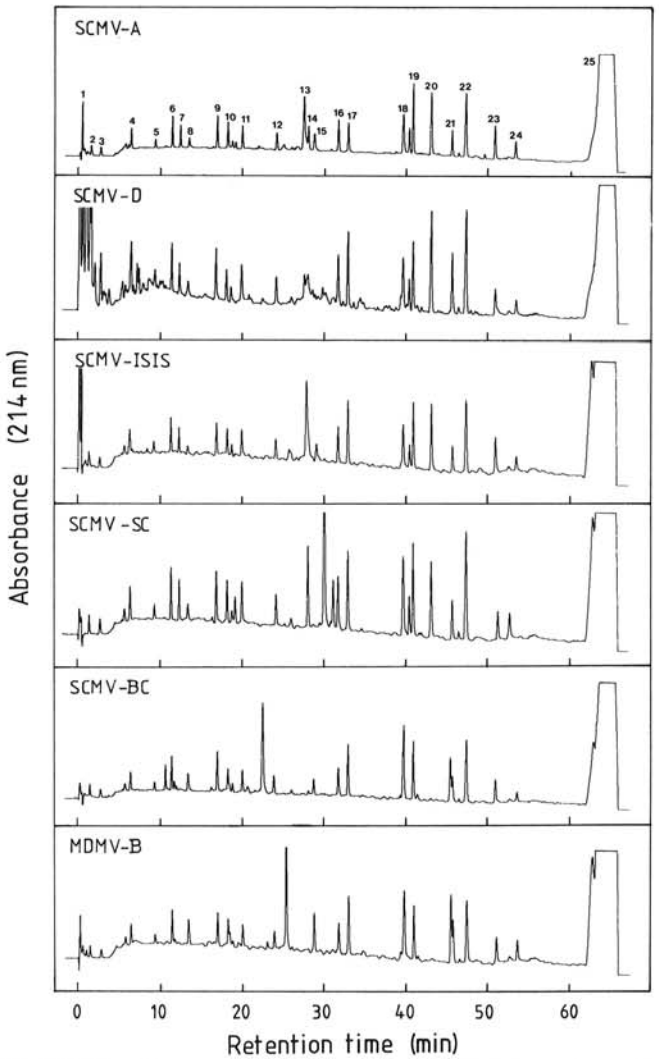

Fig. 1. High-performance liquid chromatographic (HPLC) peptide profiles of tryptic digests of coat protein from sugarcane mosaic virus (SCMV) strains SCMV-A, SCMV-D, SCMV-Isis, SCMV-SC, SCMV-BC, and maize dwarf mosaic virus (MDMV) strain MDMV-B. Conditions as described in text. First and last peaks in each profile (numbers 1 and 25 in SCMV-A) were not included in comparisons. Variation was observed in peak heights of peaks eluting late in the chromatography (after 50 min), but this could be minimized by warming digest mixtures to 45 C before loading samples on the HPLC column.

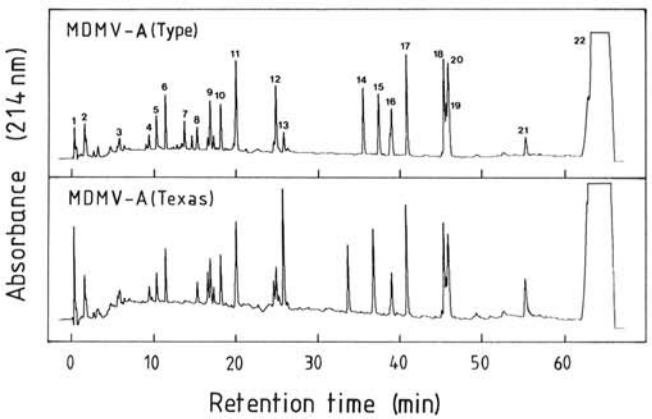

Fig. 2. High-performance liquid chromatographic peptide profiles of tryptic digests of coat protein of maize dwarf mosaic virus (MDMV) strains MDMV-A (type) and MDMV-A (Texas). Conditions as described in text. First and last peaks in each profile (numbers 1 and 22 in MDMV-A [type]) were not included in comparisons.

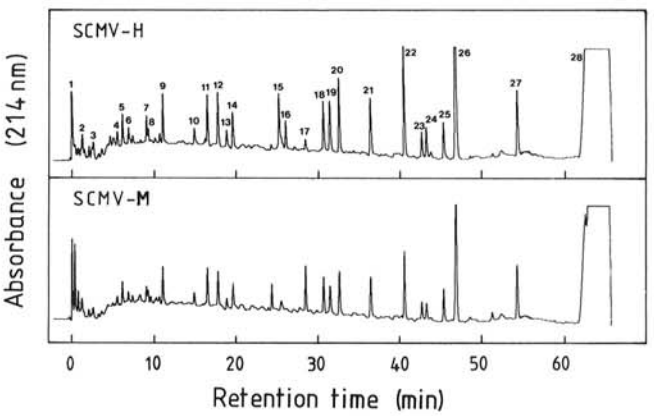

Fig. 3. High-performance liquid chromatographic peptide profiles of tryptic digests of coat protein of sugarcane mosaic virus (SCMV) strains SCMV-H and SCMV-M. Conditions as described in text. First and last peaks in each profile (numbers 1 and 28 in SCMV-H) were not included in comparisons.

of peaks from coat protein profiles of strains, examination of peptide profiles from eight distinct potyviruses—bean yellow mosaic virus, clover yellow vein virus, JGMV, peanut stripe virus, PWV, PVY, tobacco etch virus, and watermelon mosaic virus 2 (14, unpublished data)—showed that an average of 29 ± 6% of peaks (range 16–42%) had common retention times in the 28 pairwise comparisons of profiles (data not shown).

The presumption that coat protein identity reflects taxonomic relationship of potyvirus isolates has an empirical basis. In the overwhelming majority of cases where sequence data is available, it has been shown that strains classified as belonging to the one potyvirus (based on serology, host range, symptomatology, and other parameters) have coat protein sequences that have identities of approximately 90% or more. In contrast, viruses that have been shown by serological and biological parameters to be appropriately classified as distinct potyviruses have coat proteins that are substantially lower in identity. Those few isolates whose assignments based on coat protein sequence identities are at odds with the assignments based on serological properties have been reexamined. Sequence data has shown that the apparent conflict in assignment can frequently be explained by the occurrence of a common epitope of several amino acids within the N-terminal region of otherwise only distantly related coat protein sequences (13,21,22).

The results reported in this paper of HPLC peptide profiles of coat proteins from 13 strains from the SCMV group show that they fall into four groups (Table 3), which are identical to the groupings proposed by Shukla et al (18) based on reactions of coat proteins with cross-absorbed antibodies. Peptide profiling not only has confirmed that SCMV-JG and MDMV-O are members of one subgroup, designated JGMV, but has shown that MDMV-KS1 is also a strain of JGMV (9).

The substantial similarity of peptide profiles of SCMV-A, SCMV-D, SCMV-SC, SCMV-Isis, SCMV-BC, and MDMV-B (at least 70% of peaks common to all profiles—Table 2), indicates that SCMV-A, SCMV-D, SCMV-SC, SCMV-Isis, SCMV-BC, and MDMV-B are strains of the same potyvirus. This result correlates with the serological data of Shukla et al (18) and is also in agreement with limited sequence information, which demonstrates the strain relationship of SCMV-SC, SCMV-BC, and MDMV-B (2). When retention times of these six SCMV strains were compared with those from the other peptide profiles, the percentage similarity was within the range encountered for distinct potyviruses, except for comparisons with the SrMV strains SCMV-H and SCMV-M (Table 2). In these cases the percentage of peaks with similar retention times was marginally greater than that observed in the comparisons of distinct potyviruses (see earlier discussion). The greater similarity of coat protein structure that is indicated by this result correlates with the observation that the coat proteins of SCMV-SC and SrMV-H have sequence identities at the high end of the range observed for distinct potyviruses (5,26), especially within the protein core. These results may be interpreted as suggesting that the SCMV and SrMV groups have evolved relatively recently from a common ancestor.

The higher degree of similarity of SCMV-A, SCMV-D, SCMV-Isis, and SCMV-SC profiles to each other compared with their similarity to SCMV-BC and MDMV-B (Table 2) suggests that the SCMV subgroup defined by Shukla et al (18) may be further subdivided into two subsets. In this respect it is of interest to note that whereas SCMV-A, SCMV-D, SCMV-Isis, and SCMV-SC infect sugarcane, SCMV-BC and MDMV-B do not. Furthermore, Tosic et al (25) have reported that when a number of strains of the SCMV subgroup were tested for their reactivity to differential sorghum lines on the basis of symptomatology, SCMV-BC and MDMV-B formed one subset, while other SCMV strains formed another.

It has been shown in other plant virus groups that the coat protein is involved in virulence and infectivity (6,11). If a similar relationship also exists for potyviruses, then it can be speculated that the differential host range noted above for the SCMV strains may at least in part be due to coat protein structural differences, which are reflected in their sequences (11). Furthermore, Frenkel et al (2) have shown that the only substantial difference in the coat protein sequences of SCMV-SC and MDMV-B occur over a span of about 50 amino acids, beginning at position 27 from the amino terminus. Therefore it is possible that this region contains structural motifs that confer host range specificity (11). Amino terminal sequence data from other strains of the SCMV group may help in determining the validity of this hypothesis.

The percentage similarity (79%) of peptide profiles of coat protein from MDMV-A (type) and MDMV-A (Texas), considered to be isolates of MDMV-A, was within the range encountered for strains (Fig. 3 and Table 2). The observed differences in retention times of 20% of peaks from these two isolates demonstrates that there is some diversity in their coat protein structures and raises the possibility that they should be considered as different strains. The MDMV-A isolates showed much less similarity to other strains examined in this paper than to each other, in most instances within the range encountered for distinct potyviruses (16–42% similarity). The slightly greater similarity to profiles of the SrMV strains (SCMV-H and SCMV-M) is probably due to the greater than usual sequence similarity of the coat protein core that is observed between MDMV-A and SrMV-H (26) These

TABLE 3. Grouping of maize dwarf mosaic virus and sugarcane mosaic virus strains on the basis of peptide profiles of coat proteins[a]

JGMV[b]	MDMV[c]	SCMV[d]	SrMV[e]
JGMV-JG	MDMV-A type	MDMV-B	SCMV-H
(MDMV-KS1)	(MDMV-A Texas)	SCMV-A	SCMV-M
MDMV-O		SCMV-BC	
		SCMV-D	
		(SCMV-Isis)	
		SCMV-SC	

[a]Strains shown in parentheses were not previously grouped by Shukla et al (9) using cross-absorbed virus-specific antibodies.
[b]JGMV = Johnsongrass mosaic virus.
[c]MDMV = Maize dwarf mosaic virus.
[d]SCMV = Sugarcane mosaic virus.
[e]SrMV = Sorghum mosaic virus.

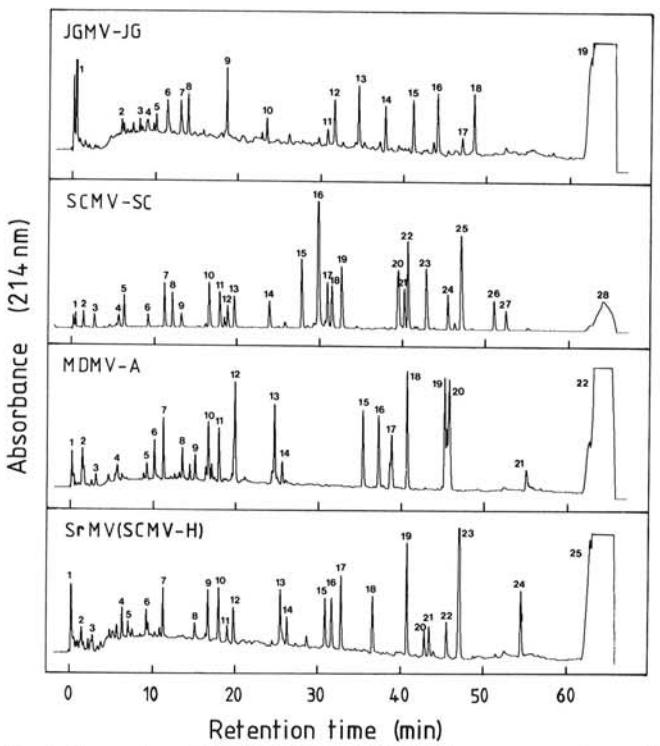

Fig. 4. Comparison of high-performance liquid chromatographic peptide profiles from Johnsongrass mosaic virus strain JG (JGMV-JG), sugarcane mosaic virus strain SC (SCMV-SC), maize dwarf mosaic virus strain A (MDMV-A), and SCMV-H. First and last peaks in each profile were not included.

results indicate that the MDMV-A isolates should be considered distinct potyviruses, as was concluded from the recent serological data (18).

The finding that retention times of 79% of peaks in the peptide profile of SCMV-H were similar to those of the SCMV-M profile (Fig. 3 and Table 2) within the range observed for strains of a potyvirus suggests that the amino acid sequences of their coat proteins are very similar and that SCMV-H and SCMV-M are strains of the one potyvirus. The same conclusion was reached by Shukla et al (18) based on reactivities of cross-absorbed virus-specific antibodies, which also demonstrated that the two strains were quite distinct in their reactivity from all of the other 15 strains tested, except for SCMV-I. Peptide profiling confirms this result, although the finding that up to 50% of SCMV-H or SCMV-M peaks had retention times similar (Table 2) to those from members of the SCMV and MDMV groups (9) indicates considerable similarity in a substantial region (probably the core) of these two coat proteins, as described earlier.

The observations made with MDMV, SCMV, and SrMV strains serve to illustrate the difficulties that may be encountered if undue reliance is placed on just a single technique when taxonomic relationships of potyvirus strains are being investigated. The clear-cut distinction that is observed between strains of these three potyviruses on the basis of reactivities of cross-absorbed virus-specific antibodies, compared with the degree of relatedness that emerges when peptide profiling of coat protein is used, may lead to different interpretations as to their relationship. The sequence data explains this apparent conflict, since the antibodies are specific for the quite different amino-terminal sequences, whereas peptide profiling also includes regions of the coat protein cores, which have substantial identity.

A similar observation has been made with another potyvirus, peanut stripe virus (PStV), whose core sequence is greater than 80% identical to sequences of strains of the distinct potyviruses PWV, watermelon mosaic virus 2, and zucchini yellow mosaic virus (8). Nevertheless, these values are still considerably less than those observed in comparisons of protein cores from strains of potyviruses (26), indicating that they are in fact distinct potyviruses. These cases probably reflect relatively recent evolutionary divergence from a common ancestor, although biological and molecular properties indicate that the viruses have diverged to a stage where they now possess the characteristics that qualify them as distinct potyviruses (see 26).

The studies reported in this paper confirm the assignment of 11 SCMV strains into four distinct potyviruses (9,18), and assign two additional strains, MDMV-A (Texas) and SCMV-Isis, to these groups. They substantiate the value of peptide profiling of potyvirus coat proteins as a technique for assisting in the clarification of taxonomic relationships between potyvirus isolates in those cases where biological and serological properties are unable to provide unambiguous answers.

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