Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 8192–8196, November 1989
Biochemistry

Localization of virus-specific and group-specific epitopes of plant potyviruses by systematic immunochemical analysis of overlapping peptide fragments

(coat proteins/synthetic peptides/epitope analysis)

D. D. SHUKLA*, G. TRIBBICK†, T. J. MASON†, D. R. HEWISH*, H. M. GEYSEN†, AND C. W. WARD*

*Commonwealth Scientific and Industrial Research Organization, Division of Biotechnology, Parkville Laboratory, 343 Royal Parade, Parkville 3052, Australia; and †Coselco Mimotopes Pty. Ltd., Corner of Duerdin and Martin Streets, Clayton 3168, Australia

Communicated by Robert J. Shepherd, June 12, 1989 (received for review November 7, 1988)

ABSTRACT **Virus-specific or group-specific antibody probes to potyviruses can be produced by targeting the immune response to the virus-specific, N-terminal region of the capsid protein (29–95 amino acids depending on the virus) or to the conserved core region (216 amino acids) of the capsid protein, respectively. Immunochemical analysis of overlapping, synthetic octapeptides covering the capsid protein of the Johnsongrass strain of Johnsongrass mosaic virus (JGMV-JG) has delineated the peptide sequences recognized by five polyclonal rabbit antisera and two mouse monoclonal antibodies (mAbs). The antibodies characterized were (*i*) three virus-specific rabbit polyclonal antisera and one virus-specific mouse mAb (1/25) raised against native virus particles, (*ii*) one polyclonal antiserum raised against trypsin-derived core particles of JGMV-JG, (*iii*) one group-specific polyclonal antiserum raised against the denatured, truncated coat protein from trypsin-derived core particles of JGMV-JG, and (*iv*) one group-specific mouse mAb (1/16) raised against native virus particles. The two epitopes seen by mAb 1/25 occurred at residues 18–27 and 43–52 and overlapped with the two major epitopes seen by the virus-specific polyclonal antiserum. The group-specific epitope seen in JGMV-JG by mAb 1/16 was also recognized strongly in potato virus Y, the type member of the potyvirus group. The multiple epitopes seen by the cross-reactive polyclonal antisera were distributed across the entire core region of the coat protein and their relative antibody binding responses varied between JGMV-JG, potato virus Y, and six other distinct potyviruses.**

Plant diseases are estimated to be responsible for economic losses worldwide of $60 billion per annum (1). The most important pathogens are fungi, with plant viruses the second most important group of infectious agents. Of the 28 plant virus groups or families the potyvirus group is the largest. It contains at least 175 independent members and accounts for about 30% of all viruses known to infect plant species around the world (2, 3). Potyvirus particles are flexuous rods, 729–900 nm long and ≈11 nm in diameter, and consist of up to 2000 subunits of a single protein species (4).

Successful control and eradication of plant virus infections is dependent on the availability of simple, reliable methods for plant virus detection and identification. To date, this has been difficult to achieve for potyviruses due to the large size of the group, the vast variation between members, and the lack of satisfactory taxonomic parameters to distinguish independent viruses from related strains (2–4). Thus, there is a real need to evaluate new criteria for the identification and classification of potyviruses.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

During investigations on the structural characterization of the coat proteins of potyviruses we made the following observations that have implications for potyvirus detection and classification: (*i*) distinct potyviruses exhibit coat protein sequence homology of 38–71% with major differences in the length (29–95 residues) and sequence of the N-terminal portion of their coat proteins but high sequence homology (65%) in the C-terminal three-quarters of the coat proteins (5); (*ii*) strains of individual viruses exhibit very high sequence homology (90–99%) (5–8); (*iii*) the N and C termini of the coat proteins are surface-located as found for other rod-shaped plant viruses such as tobamoviruses, potexviruses, and tobraviruses and can be removed from virions by mild proteolysis (8, 9).

These observations suggested that antibodies produced against the highly conserved core region of the coat protein should be excellent broad-spectrum probes capable of detecting most, if not all, potyviruses, whereas antibodies directed to the unique surface-located, N-terminal regions of the coat proteins should be highly specific and only react with strains of the one virus (8).

In this paper we describe the characterization of five rabbit polyclonal antisera and two mouse monoclonal antibodies (mAbs) targeted to different regions of the coat protein of the Johnsongrass strain of Johnsongrass mosaic virus (JGMV-JG). The virus infects the perennial weed Johnsongrass (*Sorghum halepense*) as well as the commercial crops such as sorghum (*Sorghum bicolor*) and maize (*Zea mays*). Immunochemical analysis of overlapping synthetic peptides has allowed us to delineate epitopes in JGMV-JG and establish the extent to which these epitopes are conserved across other potyviruses. Such systematic mapping was first described by Geysen *et al.* (10) in their analysis of the antigenic regions of foot and mouth disease virus coat protein VP1 and is ideally suited to the identification of sequence-dependent linear epitopes that are the targets of diagnostic reagents.

MATERIALS AND METHODS

Viruses. Potyviruses used in this study were bean yellow mosaic virus (BYMV-S), JGMV-JG, maize dwarf mosaic virus O (MDMV-O, a strain of JGMV) (11), passionfruit woodiness virus (PWV-TB), potato virus Y (PVY-D), soybean mosaic virus (SMV-V) and tobacco etch virus (TEV-HAT). The strains of the viruses used, their propagation hosts, and the methods of their purification were those described previously (12, 13).

Abbreviations: JGMV-JG, Johnsongrass strain of Johnsongrass mosaic virus; BYMV-S, bean yellow mosaic virus; MDMV-O, maize dwarf mosaic virus O; PWV-TB, passionfruit woodiness virus; PVY-D, potato virus Y; SMV-V, soybean mosaic virus; TEV-HAT, tobacco etch virus; PPV, plum pox virus; mAb, monoclonal antibody.

Antisera. Five rabbit polyclonal antisera (8, 14) and two mouse mAbs were used. Three of the polyclonal antisera, JGMV AS-1, JGMV AS-2, and JGMV AS-3, were first, second, and third bleedings, respectively, of the same rabbit given a series of injections of native particles of JGMV-JG. The injections (1 mg of virus per ml per injection) were given intravenously on days 1, 8, 15, 30, 37, and 59 with a seventh intravenous injection of a new virus preparation (2 mg of virus per ml per injection) on day 65. The three bleedings were taken on days 25, 52, and 74, respectively. The final two polyclonal antisera (referred to as JGMV core particle AS and denatured JGMV core protein AS) were raised against trypsin-derived JGMV-JG core particles and their denatured, truncated coat protein, respectively. The trypsin-derived core particles remain as assembled rods but have lost, as a result of the trypsin treatment, 69 and 18 amino acids from the N- and C-terminal ends of their coat proteins, respectively (8). The truncated coat protein was obtained by disassembly and denaturation of the trypsin-derived core particles with 60% (vol/vol) formic acid and dialysis against water (13). The antisera to trypsin-derived JGMV core particles and denatured JGMV core protein were prepared by one intramuscular injection (1 mg/ml per injection) in Freund's incomplete adjuvant on day 1; this was followed by three further intravenous injections (1 mg/ml per injection) on days 15, 29, and 43. Blood was collected 10 days after the last injection. The mAbs were produced to intact native particles of JGMV-JG (D.R.H., S. L. Tracy, and D.D.S., unpublished results). BALB/c mice were immunized by intraperitoneal injection of 50 μg of purified JGMV-JG particles in Freund's complete adjuvant; this was followed 14 days later by an intravenous injection of 50 μg of purified JGMV-JG particles in saline 4 days before cell fusion. Cell fusions, growth of hybridoma cells, cloning hybridomas, and screening for antibody production and antibody isotype were carried out as described (15).

Western Blotting. Western blotting was performed with freeze-dried coat protein preparations of the potyviruses and those of potato virus X and tobacco mosaic virus using nitrocellulose strips and horseradish peroxidase-conjugated second antibodies according to the manufacturer's directions (Bio-Rad) as described (13, 16).

Synthesis and Immunochemical Analysis of Peptides. All possible overlapping octapeptides as defined from the sequences of JGMV-JG and PVY-D (17, 18) were synthesized on acrylic acid-coated polyethylene rods and tested for antibody binding by ELISA as described by Geysen *et al.* (19).

RESULTS

Contrasting Specificity of Antibody Preparations. As shown in Fig. 1*a* JGMV AS-2 raised against native virus particles of JGMV-JG was highly specific and reacted only with the two strains of JGMV. No reaction was observed with the coat proteins of the five other potyviruses. The mouse mAb 1/25 showed higher specificity than JGMV AS-2 on Western blots and reacted only with the JG strain of JGMV and not with the MDMV-O strain (data not shown). The JGMV core particle AS gave similar reactivity patterns on Western blots to that of the JGMV AS-2 but differed in showing weak, but significant, reactions with some of the other viruses tested (Fig. 1*b*). The mouse mAb 1/16 produced in the same fusion as mAb 1/25 (Fig. 1*d*), and the denatured JGMV core protein AS (Fig. 1*c*) exhibited the broadest specificities and reacted with coat proteins of all potyviruses tested. None of the polyclonal antisera and the mAbs reacted with plant proteins or rod-shaped viruses (potato virus X and tobacco mosaic virus) belonging to other plant virus groups (results not shown).

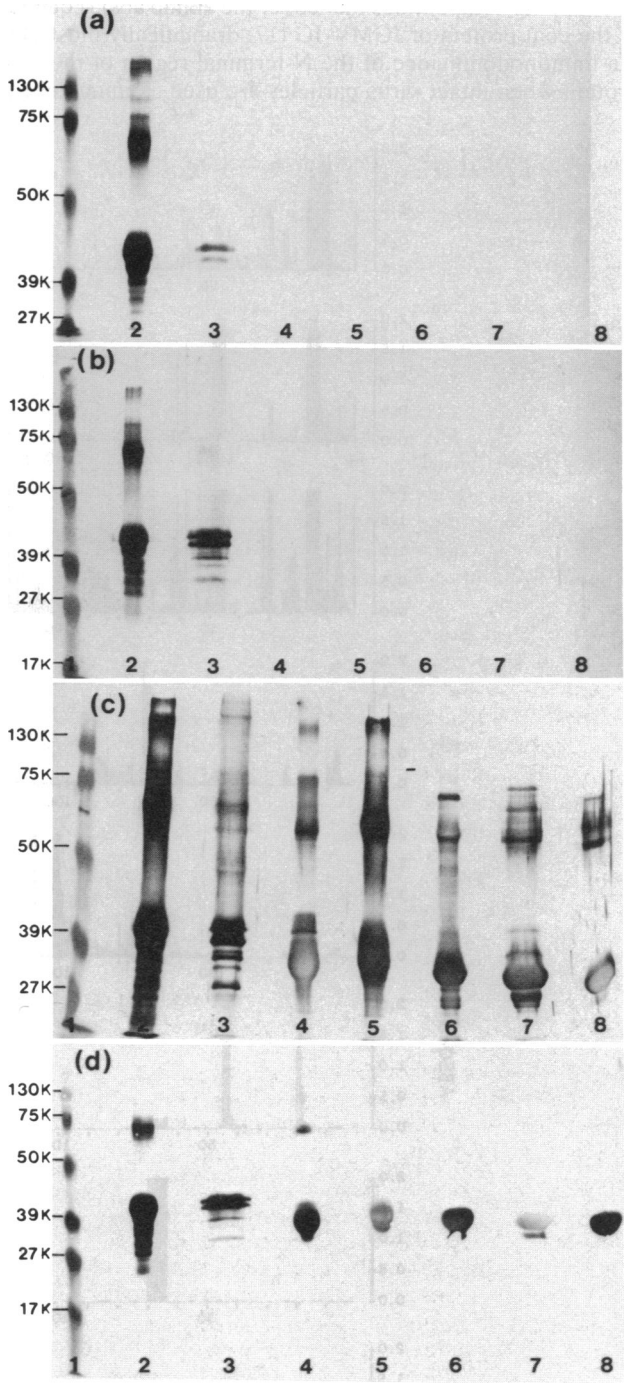

FIG. 1. Western blots of six distinct potyviruses. Approximately 5 μg of freeze-dried protein from native virus particles, dissolved in Laemmli sample buffer and heated for 3 min in boiling water, was loaded per slot of the gel. (*a*) Reaction with JGMV-JG AS-2. (*b*) Reaction with JGMV core particle AS. (*c*) Reaction with denatured JGMV core protein AS. (*d*) Reaction with mAb 1/16 produced to native JGMV-JG particles. Lanes 1, prestained Bio-Rad standards; lanes 2–8, JGMV-JG, MDMV-O strain of JGMV, BYMV-S, PWV-TB, PVY-D, SMV-V, and TEV-HAT, respectively. The multiple bands represent higher oligomers and degradation products (8). As revealed by Coomassie blue staining of the gels after transfer, only less than half of the coat proteins were actually transferred onto nitrocellulose under the electrophoretic conditions used. Coomassie-stained patterns are similar to the pattern shown in *c*. Molecular weights are shown on the left.

Identification of Virus-Specific and Group-Specific Epitopes. Systematic immunochemical screening of the 296

overlapping octapeptides that cover the amino acid sequence of the coat protein of JGMV-JG (17) dramatically illustrates the immunodominance of the N-terminal region of the coat protein when intact virus particles are used as immunogens (Fig. 2). Antibody responses following two injections (scan 1) and five injections (scan 2) were almost exclusively restricted to the N-terminal 70 amino acids with significant responses to the central and C-terminal peptide regions occurring only

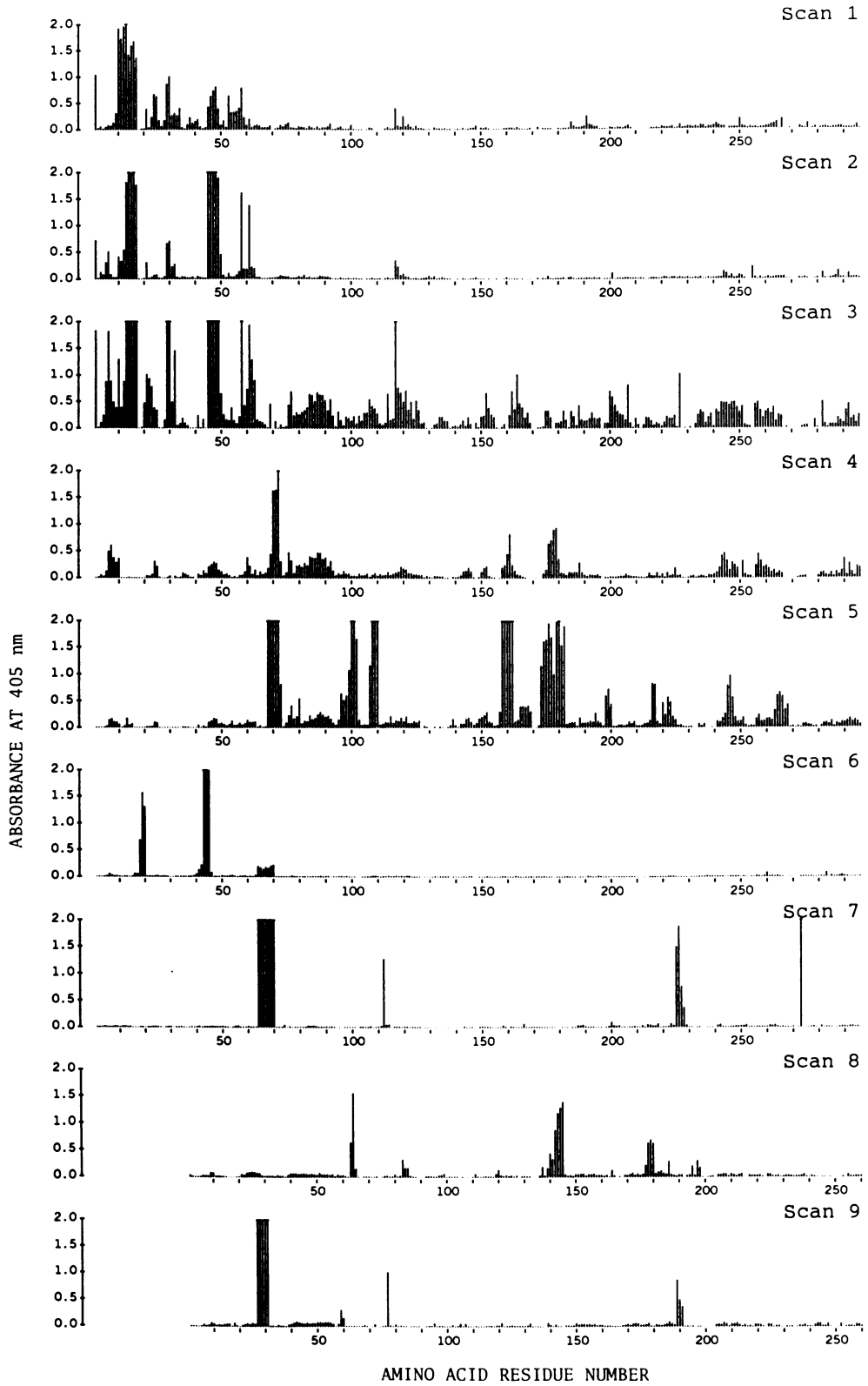

FIG. 2. Scans of antibody binding to overlapping octapeptides homologous with sequences of coat protein of JGMV (17) and PVY (18). JGMV peptides were allowed to react with: polyclonal antibodies to intact JGMV particles, JGMV AS-1 (scan 1), JGMV AS-2 (scan 2), and JGMV AS-3 (scan 3); JGMV core particle AS (scan 4); denatured JGMV core protein AS (scan 5); and mAbs 1/25 (scan 6) and 1/16 (scan 7) to intact particles of JGMV. PVY peptides were allowed to react with the denatured JGMV core protein AS (scan 8) and the mAb (1/16) to intact particles of JGMV (scan 9). The numbers along the horizontal axis refer to the first residue of each octapeptide.

after prolonged antigen boosting (scan 3). As shown in scans 1 and 2, the major virus-specific epitopes for JGMV-JG recognized by the early bleeding polyclonal antisera were residues 13–24 and 45–56 with minor epitopes at 1–8, 29–37, and 58–68 (Fig. 2). These regions are either absent or bear negligible sequence homology with the corresponding regions of other potyvirus coat proteins (5, 20) and overlapped substantially with the two epitopes (residues 18–27 and 43–52) seen by the highly specific mAb, 1/25 (Fig. 2, scan 6).

The JGMV core particle AS, though showing similar Western blot reactions to JGMV AS-2 (Fig. 1 *a* and *b*), displayed a totally different epitope specificity (Fig. 2, scan 4). This JGMV core particle AS showed strong recognition of only one peptide region, residues 70–79 with weak responses at 160–168 and 176–186 and minor responses at a few other regions (Fig. 2, scan 4). The cross-reactive antiserum, denatured JGMV core protein AS, recognized six major and five minor epitopes in the coat protein core of JGMV-JG (Fig. 2, scan 5). The major core epitopes involved residues 68–79, 99–109, 107–117, 158–169, 173–185, and 179–189, three of which correspond to the major and minor epitopes seen by the JGMV core particle AS. The remaining two overlapping major epitopes, 99–109 and 107–117, seen by the denatured JGMV core protein AS did not elicit antibodies in the rabbit immunized with trypsin-derived core particles, a finding that is consistent with the prediction that these residues are buried in the assembled core particle structure (8).

The specificity of the cross-reacting mouse mAb 1/16 was also investigated and shown to recognize JGMV-JG peptide sequences 64–77 and 273–280, with lower responses to 112–119 and 225–235 (Fig. 2, scan 7). Region 64–77 overlaps with one of the regions (residues 58–70) recognized by the JGMV AS-2 and AS-3 (Fig. 2, scans 2 and 3), with the major epitope recognized by the JGMV core particle AS (Fig. 2, scan 4) and with one of the major epitopes seen by the denatured JGMV core protein AS (Fig. 2, scan 5). Comparison of the coat protein sequences of seven other potyviruses (20) shows these potentially cross-reactive epitopes are well conserved but not identical.

It was of interest to investigate whether all six major core eiptopes seen in JGMV-JG are recognized in other potyvirus coat proteins. The overlapping octapeptides for the amino acid sequences equivalent to JGMV-JG residues 68–79, 99–109, 107–117, 158–169, 173–185, and 179–189 (core epitopes 1–6) in seven other potyviruses were synthesized and tested for their ability to bind the cross-reactive denatured JGMV core protein AS. As shown in Fig. 3, these peptides were not all recognized in the other seven potyviruses. The major cross-reactive epitopes were core epitopes 2, 4, 5, and 6. Core epitope 2 was recognized in all potyviruses tested except SMV-V. Core epitope 4 was recognized strongly in PWV-TB and moderately in PPV-D, TVMV, SMV-V, and SMV-N, whereas core epitope 6 was readily recognized in PVY-D and SMV-N. Core epitope 5 was recognized weakly in PVY-D, PWV-TB, SMV-V, SMV-N, and PPV-D, whereas core epitope 3 was only recognized in one other virus, PWV-TB. Core epitope 1 (residues 68–79) did not score as a cross-reacting epitope with the denatured JGMV core protein AS and was not recognized in any of the other potyviruses tested except SMV-N, where the reaction was relatively minor. This was surprising, since it overlaps substantially with residues 64–77, the major epitope seen by the cross-reacting mAb 1/16, and since the amino acid residues at positions 64, 65, and 67 are not conserved in different potyviruses (20).

It was also of interest to examine whether regions corresponding to minor epitopes in JGMV-JG or additional epitopes are recognized in the coat proteins of other potyviruses. To this end the 260 overlapping octapeptides that cover the sequence of the coat protein of PVY-D (18) were

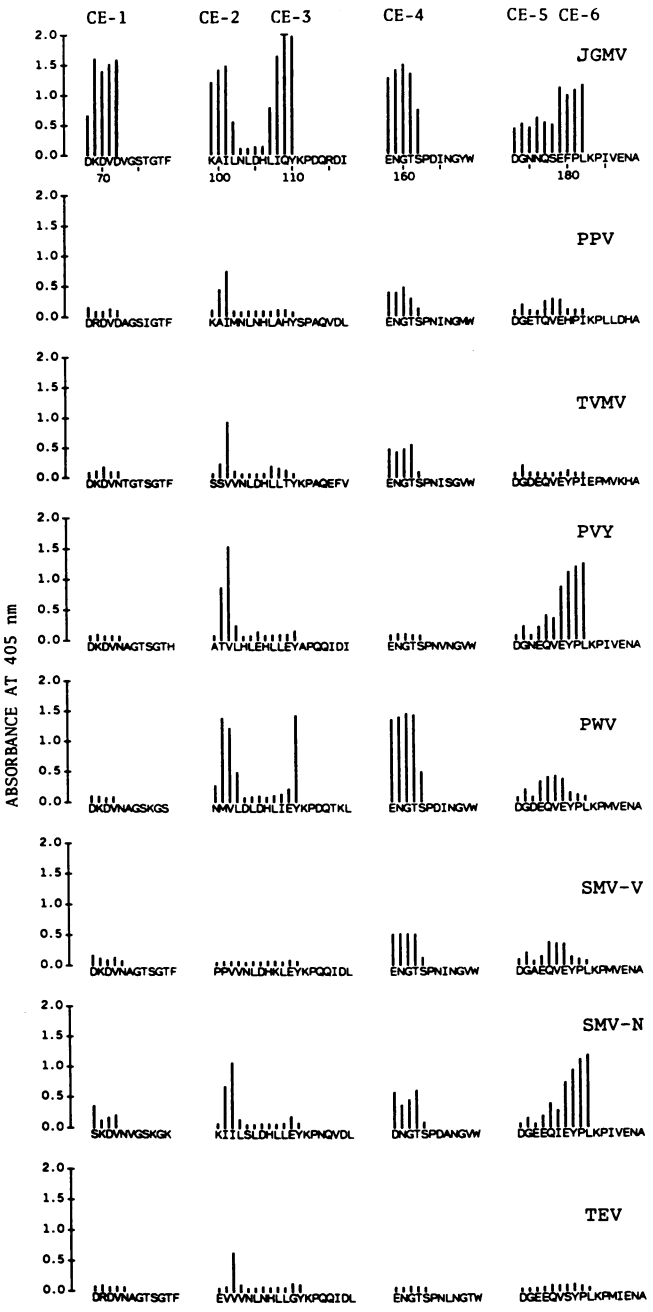

FIG. 3. Scans of antibody binding to overlapping octapeptides homologous with the coat protein sequences of eight distinct potyviruses at regions equivalent to core epitopes (CE) 1–6 (residues 68–79, 99–109, 107–117, 158–169, 173–185, and 179–189) in JGMV-JG. The seven other potyviruses and their coat protein sequences are plum pox virus (PPV-D; ref. 21); tobacco vein mottling virus (TVMV; ref. 22); PVY-D (18); PWV-TB (7); SMV-V (23); SMV-N (24); tobacco etch virus (TEV-NAT; ref. 9). The antiserum used was the rabbit polyclonal antiserum raised against the denatured truncated coat protein from trypsin-derived core particles of JGMV-JG. The antibody binding for each octapeptide is depicted above the first residue of that peptide.

synthesized and screened with the denatured JGMV core protein AS. As shown in Fig. 2, scan 8, no new major epitopes were found. The only major epitopes were those corresponding to core epitopes 2 (residues 63–71) and 6 (residues 142–152) identified in the restricted analysis (Fig. 3). Moderate binding was observed at residues 137–148 (equivalent to core epitope 5 in JGMV) and at residues 178–187 (equivalent to the minor core epitope at residues 216–224 in JGMV).

Weak responses were found at 186–193 (equivalent to a minor epitope in JGMV) and at the new positions 83–92 and 195–205, regions not recognized in JGMV.

When mAb 1/16 was tested against the PVY-D coat protein peptides, three of the four regions seen by this antibody in JGMV-JG were recognized (Fig. 2, scan 9). The major epitope (residues 27–38) is equivalent to residues 64–75 in the major epitope (core epitope 1) seen in the JGMV coat protein, whereas the minor responses at 77–84 and 189–198 overlap or are equivalent to the minor responses at 112–119 and 223–232 in JGMV, respectively (Fig. 2, scan 7). The single response with mAb 1/16 at 273–280 in JGMV was not seen with the corresponding PVY peptide.

DISCUSSION

Serology has been shown to be a very useful criterion for the identification and classification of members of several plant virus groups, notably tobamoviruses (25) and tymoviruses (26). However, to date it has proved most unsatisfactory when applied to the large potyvirus group as serological relationships between related strains and distinct members have been found to be complex and inconsistent (2–5).

We have recently shown that much of this confusion has resulted from the use of diagnostic antisera that contain variable proportions of cross-reacting antibodies directed toward the conserved core region of the virus coat protein (8, 13). The occurrence of these antibodies can be minimized by careful immunization protocols (8) or they can be removed by cross absorption (11, 13). These findings highlighted the importance of using well-characterized diagnostic reagents for plant virus detection and identification.

The epitope mapping technique employed here is a very powerful method for characterizing antibody preparations and assessing the effects of amino acid sequence changes on epitope reactivity. In this paper we have shown that the epitopes recognized by broad-spectrum cross-reactive antibodies are distributed across the entire core region of the coat protein and that their relative contribution to total antibody binding varies between different potyviruses (Figs. 2 and 3).

Of great interest was the finding that the major region recognized by the cross-reacting mouse mAb raised against whole JGMV-JG particles was the major region recognized by the polyclonal antisera to trypsin-derived core particles of JGMV-JG and corresponds to core epitope 1 recognized by the JGMV core protein antisera. The region has the highest peak of hydrophilicity in the total coat protein and is surface-located in native virus particles since it includes the two lysine residues, 67 and 69, which mark the junction between the trypsin-susceptible N-terminal region and the trypsin-resistant core (8).

The different recognition of the core epitope sequences from different potyviruses (Fig. 3) and the reactivity of the mAbs 1/25 and 1/16 for multiple epitopes (Fig. 2) may reflect previous observations that many of the residues in linear epitopes can be substituted and that only a few of the amino acids are key contact residues (10, 19). Also, it is interesting to note that mAbs have been found to bind to proteins that display no significant sequence homology (27, 28).

In this report we have also shown that the epitopes recognized by the virus-specific mAbs and polyclonal antibodies are linear sequences located in the N-terminal region (residues 1–67) of the coat protein, which is known to be exposed on the surface of native virus particles (8, 9). Surprisingly, these virus-specific reagents contained no antibodies to peptide sequences in the C-terminal 18-amino acid residues, which can also be removed from native virus particles by trypsin treatment (8), or to the conserved core region of the coat protein. The proportion of antibodies directed toward conformational epitopes in native virus particles is not known, but these antibodies would be expected to play a minor role in peptide binding and Western blot reactions.

The systematic immunochemical analysis technique employed here has the potential to resolve many of the problems currently associated with potyvirus serology. It should be possible to identify the epitopes responsible for the unexpected and inconsistent "paired" relationships reported between independent potyviruses (2–5, 8, 13) and to establish the cause for the very poor serological cross-reactivity between pepper mottle virus and strains of PVY despite their very close sequence homology (3, 6). In turn it should lead to the use of synthetic peptides corresponding to defined epitopes, to generate virus-specific and group-specific serological probes.

In view of the structural similarities in polypeptide folding and subunit packing among rod-shaped plant viruses (8), our results with potyviruses may have relevance for members in the carla-, clostero-, potex-, tobamo-, and tobravirus groups.

We are most grateful to Dr. R. N. Beachy (Washington University, Saint Louis), Dr. S. A. Tolin (Virginia Polytechnic Institute and State University, Blacksburg), and Dr. M. Ravelonandro (Station de Pathologie Vegetale, Institut National de la Recherche Agronomique, Pont-de-la-Maye, France) for their generous provision of sequence data on SMV-N, SMV-V, and PPV, respectively, prior to publication. We thank Ms. S. L. Tracy, Mrs. H. Gould, and Mr. S. Laurie for excellent technical assistance. This work was supported by the Rural Credits Development Fund of the Reserve Bank of Australia.

1. Klausner, A. (1987) *Biotechnology* **5**, 551–556.
2. Francki, R. I. B., Milne, R. G. & Hatta, T. (1985) *Atlas of Plant Viruses* (CRC, Boca Raton, FL), Vols. 1 and 2.
3. Shukla, D. D. & Ward, C. W. (1989) *Arch. Virol.* **106**, 171–200.
4. Hollings, M. & Brunt, A. A. (1981) in *Handbook of Plant Virus Infections: Comparative Diagnosis*, ed. Kurstak, E. (Elsevier/North Holland Biomedical, New York), pp. 731–807.
5. Shukla, D. D. & Ward, C. W. (1988) *J. Gen. Virol.* **69**, 2703–2710.
6. Shukla, D. D., Thomas, J. E., McKern, N. M., Tracy, S. L. & Ward, C. W. (1988) *Arch. Virol.* **102**, 207–219.
7. Shukla, D. D., McKern, N. M. & Ward, C. W. (1988) *Arch. Virol.* **102**, 221–232.
8. Shukla, D. D., Strike, P. M., Tracy, S. L., Gough, K. H. & Ward, C. W. (1988) *J. Gen. Virol.* **69**, 1497–1508.
9. Allison, R. F., Sorenson, J. C., Kelly, M. E., Armstrong, F. B. & Dougherty, W. G. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 3969–3972.
10. Geysen, H. M., Meloen, R. H. & Barteling, S. J. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 3998–4002.
11. Shukla, D. D., Tosic, M., Jilka, J., Ford, R. E., Toler, R. W. & Langham, M. A. C. (1989) *Phytopathology* **79**, 223–229.
12. Gough, K. H. & Shukla, D. D. (1981) *Virology* **111**, 455–462.
13. Shukla, D. D., Jilka, J., Tosic, M. & Ford, R. E. (1989) *J. Gen. Virol.* **70**, 13–23.
14. Shukla, D. D. & Gough, K. H. (1979) *J. Gen. Virol.* **45**, 533–536.
15. Hewish, D. R., Sward, R. J., Shukla, D. D. & Rochow, W. F. (1987) *APP Australas. Plant Pathol.* **16**, 1–4.
16. O'Donnell, I. J., Shukla, D. D. & Gough, K. H. (1982) *J. Virol. Methods* **4**, 19–26.
17. Shukla, D. D., Gough, K. H. & Ward, C. W. (1987) *Arch. Virol.* **96**, 59–74.
18. Shukla, D. D., Inglis, A. S., McKern, N. M. & Gough, K. H. (1986) *Virology* **152**, 118–125.
19. Geysen, H. M., Rodda, S. J., Mason, T. J., Tribbik, G. & Schoofs, P. G. (1987) *J. Immunol. Methods* **102**, 259–274.
20. Shukla, D. D. & Ward, C. W. (1989) *Adv. Virus Res.* **36**, 273–314.
21. Ravelonandro, M., Varveri, C., Delbos, R. & Dunez, J. (1988) *J. Gen. Virol.* **69**, 1509–1516.
22. Domier, L. L., Franklin, K. M., Shahbuddin, M., Hellman, G. M., Overmeyer, J. H., Hiremath, S. T., Siaw, M. E. E., Lomonossoff, G. P., Shaw, J. G. & Rhoads, E. (1986) *Nucleic Acids Res.* **14**, 5417–5430.
23. Gunyuzlu, P. L., Tolin, S. A. & Johnson, J. L. (1987) *Phytopathology* **77**, 1766.
24. Eggenberger, A. L., Stark, D. M. & Beachy, R. N. (1989) *J. Gen. Virol.* **70**, 1853–1860.
25. Gibbs, A. J. (1977) *Commonw. Mycol. Inst./Assoc. Applied Biol. Descrip. Plant Vir.*, No. 84.
26. Koenig, R. & Lessemann, D. E. (1979) *Commonw. Mycol. Inst./Assoc. Applied Biol. Descrip. Plant Vir.*, No. 214.
27. Angello, V., Arbetter, A., Ibanez de Kasep, G., Powell, R., Tan, E. M. & Joslin, F. (1980) *J. Exp. Med.* **151**, 1514–1527.
28. Ogata, C., Hadata, M., Tomlinson, G., Shin, W.-C. & Kim, S.-H. (1987) *Nature (London)* **328**, 739–742.