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# Peptide Electrophoretic Patterns of Cucumoviruses

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## ABSTRAK

*Corak elektroforesis peptid daripada beberapa pencilan cucumovirus telah dibandingkan dengan menggunakan 15% gel poliakrilamid berkeping. Kedua-dua protease dari Staphylococcus aureus V8 dan α-chymotrypsin telah menghadamkan, secara separa, subunit-subunit protein dari virus-virus ini kepada peptid-peptid yang membentuk corak-corak yang berlainan apabila dielektroforesiskan melalui gel-gel tersebut. Perbezaan-perbezaan yang nyata telah dicerap di antara corak-corak elektroforesis peptid untuk virus-virus* cucumber mosaic, peanut stunt *dan* tomato aspermy. *Jika pun ada perbezaan-perbezaan di antara strain-strain virus yang sama, ia tidak ketara atau tidak nyata.*

## ABSTRACT

*The peptide electrophoretic patterns of several cucumovirus isolates were compared using 15% polyacrylamide slab-gels. Both Staphylococcus aureus V8 protease and α-chymotrypsin partially digested the protein subunits of these viruses into peptides which produced different patterns when electrophoresed though the gels. Major differences were observed among peptide electrophoretic patterns of cucumber mosaic, peanut stunt and tomato aspermy viruses. Differences, if they do exist, among strains of the same virus were either inapparent or minor.*

## INTRODUCTION

Several isolates of cucumoviruses have been isolated and identified as cucumber mosaic virus (CMV), peanut stunt virus (PSV), and tomato aspermy virus (TAV) (Ahmad and Scott, 1984; 1985; 1986; Ahmad, 1987). These isolates were differentiated from one another and grouped according to symptoms induced on several test plants and their serological characteristics (Ahmad and Scott, 1985). Data from these studies have shown that all the CMV isolates studied belong to the CMV-DTL serogroup, and that all the PSV isolates belong to the PSV-W serogroup (Devergne and Cardin, 1973. 1976).

Edwards and Gonsalves (1983) have shown that two distinct groups of CMV isolates could be elucidated from electrophoretic patterns of seven CMV isolates. While Edwards and Gonzalves (1983) used only CMV isolates collected in New York, United States, the present study attempts to include other cucumoviruses, that is PSV and TAV, plus CMV isolates which originated from France (Marchoux *et al.*, 1973) and South Africa (Van Regenmortel *et al.*, 1972). In this

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paper we present data which add evidence of the usefulness of this technique for comparing isolates of CMV and for differentiating viruses belonging to the cucumovirus group.

## MATERIALS AND METHODS

*Preparation of Viral Protein Subunits*

The viruses and their strains used for the study were obtained, propagated and purified as reported previously (Ahmad and Scott 1985, 1986). Brome mosaic virus (BMV) which was used for comparison were purified according to Scott and Slack (1971). An equal volume of glycerol was added to purified virus preparations which were then stored at −20°C until used (Fukumoto and Tochihara, 1980).

Purified cucumoviruses and BMV were subjected to a high-speed centrifugation at 30,000 for 150 minutes in a Beckman L3 ultracentrifuge. The pellet was resuspended in distilled water and the concentration was adjusted to 2.5 mg/ml virus. An equal volume of double-strength disscociation buffer (Cleveland *et al.*, 1977) was added to the virus suspension and the mixture was then boiled for 2 minutes. This procedure yielded 1 mg/ml viral proteins (assuming that the virion contains 20% RNA) in 0.125 M Tris-HCI buffer, pH 6.8, 0.1% sodium dodecyl sulphate (SDS), 10% glycerol and 0.001% bromophenol blue dye (Cleveland *et al.*, 1977).

*Limited Proteolysis of Protein Subunits*

Limited proteolysis of CMV, PSV and TAV protein subunits was carried out as described by Tijssen and Kurstak (1981) with minor modifications. Ten microliters of 600 ug/ml *Staphylococcus aureus* V8 enzyme (SAV8) (Mills laboratory, Elkhart, I11, USA) were added to 50 µl of 1 mg/ml viral protein samples giving a final concentration of 100 µg/ml enzyme in the reaction mixture. The enzymatic reaction was maintained at 37°C and was allowed to proceed for 2 hours, after which it was stopped by adding 10 µl of 30% 2-mercaptoethanol-25% SDS solution and boiling for 2 minutes.

Proteolysis of protein subunits of the various cucumovirus isolates with α-chymotrypsin (Worthington, Freehold, N.J., USA) was carried by incubation with 50 µg/ml enzyme for 30 minutes. The proteolytic process was carried out in the same manner as above.

*Polyacrylamide Gel Electrophoresis*

Fifteen percent slab-gels for the separation of peptides were prepared in 0.375 M Tris-HCI buffer, pH 8.8. After an overnight incubation, 3% stacking gels prepared in 0.125 M Tris-HCI buffer, pH 6.8, were layered on top of the separation gels (Cleveland *et al.*, 1977). Wells were formed by inserting a ten-welled comb into the solidifying stacking gel layer. The gels were preelectrophoresed for 2 hours prior to the addition of 10 µl partially digested protein samples on top of the gels. Electrophoresis was then performed in a verticle slab-gel electrophoresis apparatus (Biorad Laboratories, Richmond, CA, USA) for 7 hours at a constant voltage of 40 V. The peptide bands were visualised by staining with 1% Coomassie blue dye in 50% methanol-10% acetic acid, and then destaining with 5% methanol-10% acetic acid (Cleveland *et al.*, 1977).

Polyacrylamide gel electrophoresis for the estimation of peptide molecular weights were performed according to Weber and Osborne (1969), using 7.5% cylindrical gels. After 2 hours of electrophoresis at 50 V the gels were stained and destained (Cleveland *et al.*, 1977) to visualise the peptide bands.

## RESULTS AND DISCUSSION

Each of the three cucumoviruses and BMV formed different peptide electrophoretic patterns following partial digestion with either SAV8 protease *(Fig. 1a)* or α-chymotrypsin *(Fig. 1b)*. Using the stated enzyme concentrations and time of incubation, the partial digestion process of protein subunits of most isolates into smaller peptides was completed, since the bands representing the original undigested proteins were either absent or weak. Reproducibility of this technique was demonstrated by the production

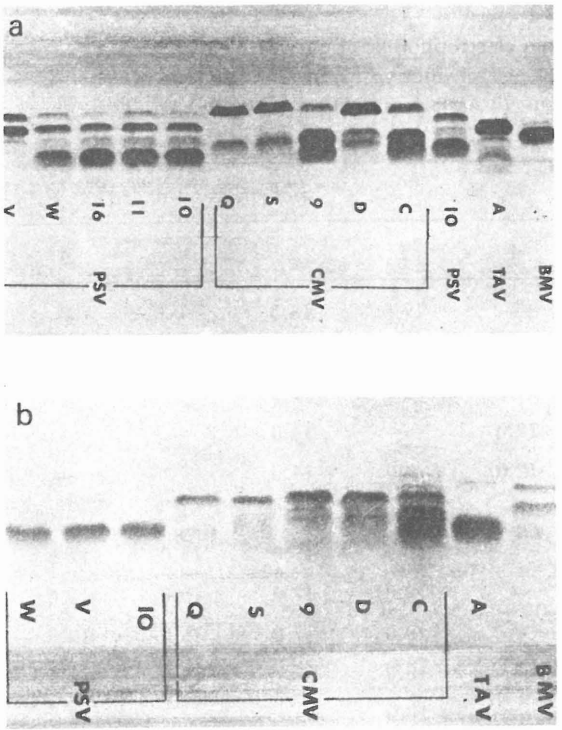


*Fig. 1: Peptide electrophoretic patterns of (a)* Staphylococcus aureus *V8 protease-cleaved or (b) α-chymotrypsin-cleaved protein subunits obtained by slab-polyacrylamide gel electrophoresis: The viruses were brome mosaic (BMV), tomato aspermy (TAV), cucumber mosaic (CMV-C, CMV-D, CMV-9, CMV-S, CMV-Q), and peanut stunt (PSV-10, PSV-11, PSV-16, PSV-W, PSV-V). The arrow indicates direction of peptide migration.*

of identical peptide maps of five different preparations each of CMV-C and PSV-10 isolates, following partial digestion with SAV8. Identical peptide maps were also demonstrated when the same preparations were partially digested with α-chymotrypsin and then subjected to electrophoresis.

Electrophoresis of α-chymotrypsin cleavage products revealed similarities in peptide electrophoretic patterns among all but Q and S isolates of CMV *(Fig. 1b)*. The sizes of peptides derived from these two isolated differed from the respective peptides of the other CMV isolates.

Different electrophoretic patterns were obtained for the three cucumoviruses using either SAV8 protease *(Fig. 1a)* or α-chymotrypsin *(Fig. 1b)* for partial digestion of protein subunits. Moreover, BMV which belongs to the bromovirus group, was shown to have a greater degree of difference in peptide electrophoretic patterns than the other three viruses.

Electrophoresis of cleavage products derived from protein subunits of different isolates of the same virus revealed peptides varying numbers, molecular size and intensity of their bands depending on the enzyme used (Table 1, *Fig. 1)*. Electrophoresis of SAV8 cleavage products showed that the size of the two slower migrating peptides of the various CMV isolates were identical, but the third slower migrating bands moved different distances. For example, this peptide migrated to a lesser distance in CMV-D, CMV-Q and CMV-S when compared to the rest of the CMV isolates *(Fig. 1)*.

Peptide electrophoretic patterns of different isolates of the same virus varied with respect to intensity. These differences were random and were not associated with any particular serotype or serogroup. This variation was difficult to resolve. On one hand, they might represent only apparent differences since enzymatic cleavage of the proteins is transient. On the other hand, differing sensitivites of the different CMV protein subunits to a particular enzyme could be due to real differences in their amino acid sequences (Koenig *et al.*, 1981). The slower rate of degradation may be due to obscurity of the susceptible bonds in the quarternary structure of the protein subunits.

The overall peptide electrophoretic patterns of CMV-Q and CMV-S differed from the rest of the CMV isolates *(Fig. 1)*. Ten other CMV isolates, namely, CMV-14, CMV-CL, CMV-J, CMV-78, CMV-61, CMV-EM, CMV-23, CMV-81, CMV-233 and CMV-DF (Ahmad and Scott, 1985) showed peptide electrophoretic patterns similar to that of CMV-C (Result not shown). Hence this study supports the contention that none of the CMV isolates studied belong to the

TABLE 1
Molecular weights of peptides obtained from electrophoresis of enzyme-cleaved (with *Staphylococcus aureus* V8 protese (SAV8) or α-chymotrypsin) protein subunits of brome mosaic virus (BMV), tomato aspermy virus (TAV), peanut stunt virus (PSV) and cucumber mosaic virus (CMV)

| | SAV8 | | | | a-chymotrypsin | | | |
|---|---|---|---|---|---|---|---|---|
| Viruses | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| BMV | 16.5 | 13.5 | 9.0 | — | 17.5 | 13.5 | — | — |
| TAV-A | 17.0 | 13.5 | 10.3 | — | 21.0 | 15.0 | — | — |
| TAV-C | 17.0 | 13.5 | 10.3 | — | 21.0 | 15.0 | — | — |
| PSV-10 | 22.0 | 18.0 | 15.0 | 12.0 | — | 14.0 | — | — |
| PSV-W | 22.0 | 18.0 | 15.0 | 12.0 | — | 14.0 | — | — |
| PSV-V | 22.0 | 18.0 | 15.0 | — | 19.0 | 14.0 | — | — |
| CMV-C | 23.0 | 15.0 | 12.0 | — | 19.0 | 17.0 | 15.0 | 12.5 |
| CMV-D | 23.0 | 15.0 | 12.5 | — | 19.0 | 17.0 | 15.0 | 12.5 |
| CMV-9 | 23.0 | 15.0 | 12.5 | — | 19.0 | 17.0 | 15.0 | 12.5 |
| CMV-S | 23.0 | — | 14.0 | — | 19.0 | — | 15.0 | 12.5 |
| CMV-Q | 23.0 | — | 13.0 | — | 19.0 | — | — | 12.5 |

CMV-ToRS serogroup except for CMV-Q and CMV-S (Ahmad and Scott, 1985).

Two isolates of TAV were tested, namely, TAV-A and TAV-C which are isolates from Arkansas, United States and Canada, respectively. The peptide electrophoretic pattern of TAV-A differed from the other viruses and could therefore be distinguished from the other viruses easily. However, the two isolates did not show any difference in peptide electrophoretic pattern when they were run together in the same gel (Table 1).

With regard to PSV, there appeared to be no observable difference among peptide electrophoretic patterns of five isolates tested. This is significant since one isolate, PSV-V belongs to the PSV-V serogroup, whereas the others, PSV-10, PSV-11, PSV-16 and PSV-W belong to the PSV-W serogroup (Ahmad and Scott, 1985). The lesser extent of cleavage in the PSV-V protein subunit resulted in the production of faster moving bands with very light intensity; this may give a false impression of pattern difference (Koenig *et al.*, 1981). It appeared therefore, that serological studies are more sensitive than electrophoresis of peptides in differentiating PSV isolates. Moreover, the close similarities in peptide electrophoretic patterns of the various isolates of the same virus are expected for three reasons: First, protein subunits of different CMV strains are serologically indistinguishable (Van Regenmortel, 1978). Second, the polyacrylamide gel electrophoresis technique gives results with about 10% accuracy (Weber and Osborne, 1969) which implies that peptides differing in few amino acids may not be distinguishable. Third, peptide bands which have migrated to a similar distance following electrophoresis may contain entirely different numbers of amino acids (Koenig *et al.*, 1981). Despite this drawback, the technique was useful for comparing and differentiating CMV strains and viruses belonging to the cucumovirus groups.

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