Serological Differentiation between Top Component and Nucleoprotein Components of Comoviruses

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SUMMARY

Rabbit antisera produced against two comoviruses (cowpea mosaic virus and cowpea severe mosaic virus) were used in plate-trapped ELISA and liquid phase competition ELISA. In the latter, the top component competed against bound unfractionated virus more effectively than did nucleoprotein components. One murine monoclonal antibody elicited to cowpea mosaic virus and three monoclonal antibodies to cowpea severe mosaic virus exhibited differential binding to top component, relative to either middle or bottom components in plate-trapped or antibody-trapped ELISAs. Differential binding to centrifugal components of virus by two of these monoclonal antibodies was maintained in liquid phase competition assays. These data suggest that the encapsidation of RNA alters the configuration of the virion surface in the vicinity of the antibody epitopes. Ribonuclease digestion of intact or denatured virus did not affect the binding of monoclonal antibodies.

Like other comoviruses, cowpea mosaic virus (CPMV) and cowpea severe mosaic virus (CPSMV) are resolved into three major fractions by density gradient centrifugation (Bruening, 1969). The slowest sedimenting top component consists of empty capsids whereas the middle and bottom components are nucleocapsids containing one molecule of either RNA 2 (M, 1.37 x 10^6) or RNA 1 (M, 2.02 x 10^6), respectively. PAGE does not show differences between the protein composition of the three components (Geelen et al., 1972; Wu & Bruening, 1971) and rabbit antisera prepared against unfractionated virus reacts with all components in Ouchterlony double-diffusion assays (Bruening & Agrawal, 1967). This paper reports that liquid phase competition assays utilizing rabbit antisera reveal a difference between the binding of antibodies to empty capsids and to nucleoprotein virions. Antigenic differences between top component and middle or bottom components were confirmed by assays with monoclonal antibodies.

CPSMV-DG and CPMV-SB isolates were originally obtained from G. Bruening (Department of Plant Pathology, University of California, Davis, Ca., U.S.A.), propagated in cowpeas (Vigna unguiculata Walp. cv. California Black Eye) and isolated as previously described (Bruening, 1969). Approximately 5 mg virus was suspended in 0.5 ml gradient buffer (50 mM-KH₂PO₄ pH 7.0, 2 mM-disodium EDTA) and layered onto 11 ml of 39° (w/w) caesium chloride in gradient buffer. Gradients were centrifuged for 36 h at 36000 r.p.m. and 4°C in an SW 40Ti rotor (Beckman) and fractionated by side-puncture. Fractions were diluted at least 10-fold in gradient buffer and pelleted by centrifugation at 45000 r.p.m. for 2 h. The pellets were resuspended in gradient buffer. Concentrations of virus components were calculated using A₂₆₀ at 1 mg/ml of 6.2, 10.0 and 8.1 for middle component, bottom component and unfractionated virus and A₂₈₀ at 1 mg/ml of 1.28 for top component (Geelen et al., 1972).

The preparation of rabbit antisera and of the panel of monoclonal antibodies produced by hybridomas have been described (Kalmar & Eastwell, 1989). Rabbit antisera R90 and R94 were
Fig. 1. A comparison of rabbit antisera binding to centrifugal components of either CPMV or CPSMV in ELISA (left panel) and liquid phase competition assays (right panel). Antigen: top component (○), middle component (●) and bottom component (■). All assays are plate-trapped ELISA.

preparation against CPSMV and CPMV, respectively. Hybridomas SB1 to SB9 and DG3 to DG11 secreted murine monoclonal antibodies that were elicited by unfractionated CPMV and CPSMV.

Three types of indirect ELISA were performed as described by Kalmar & Eastwell (1989). For antibody-trapped assays, polystyrene microtitre plates (Linbro; Flow Laboratories) were coated with rabbit antibody. In plate-trapped ELISAs, the antigen was bound directly to polyvinyl microtitre plates (Falcon). In both types of assay, the plates were washed extensively and the immobilized antigen was allowed to bind with the antibody to be tested. A liquid phase competition assay was performed to determine the relative binding of an antibody to a soluble antigen and a solid-phase antigen. In all cases, the quantity of antibody bound was detected with either goat anti-mouse-(IgG and IgM) or goat anti-rabbit-(IgG and IgM) conjugated with alkaline phosphatase (Kirkegaard & Perry Laboratories). Colour was developed with phosphatase substrate tablets (Sigma) in 10% diethanolamine pH 9.8. Absorbance values were determined (BioTek microtitre plate reader) and the reported values are the difference between readings at 405 nm and 495 nm.

Dilutions of individual virus components of CPMV or CPSMV were assayed in plate-trapped ELISA with rabbit antibodies. The results for all three virus components were collinear (Fig. 1), suggesting that each virus component binds equally to the plates and that the components bind equal amounts of rabbit antibodies. These simple dilution curves support the earlier results of double-diffusion assays, which suggested that polyclonal sera did not distinguish between centrifugal components of CPMV (Bruening & Agrawal, 1967). However, binding of antigen to microtitre plates promotes substantial changes in conformation and antigenic character of proteins (Friguet et al., 1984; Halk, 1986; Muller et al., 1986). To minimize the influence of these factors on measurement of antibody specificity, liquid phase competition assays were performed (McCullough et al., 1985). These assays revealed a gradation in binding: top > middle, bottom (Fig. 1).

Fifteen monoclonal antibodies were also tested in homologous binding assays for differential
binding to unfractionated virus or individual virus components. The majority of monoclonal antibodies bound to the unfractionated virus and the virus components with equal affinity. However, some monoclonal antibodies exhibited distinct preferences in binding to individual virus components. Homologous ELISA utilizing antibodies SB5 (Fig. 2), DG9 and DG11 (Fig. 3) yielded greater absorbance values for middle and bottom components than for top component, suggesting greater binding to virions containing RNA. In contrast, ELISA with antibody DG4 (Fig. 3) displayed a higher signal strength for top component on a molar basis. There was no significant difference between recognition of middle and bottom components by this group of monoclonal antibodies and there were no differences between the binding of antibody SB2 to top, middle or bottom components (Fig. 2).

A comparison of monoclonal antibody binding to virus components was also performed using liquid phase competition assays. A greater fraction of antibody SB5 was available for binding to the solid phase antigen when the top component, compared to middle or bottom components, was the competing soluble antigen (Fig. 2). This was consistent with the diminished binding to the top component noted in simple affinity assays. Similarly, binding of DG4 to CPSMV was inhibited at lower molar concentrations of top component and thus reflected greater binding to empty capsids (Fig. 3). This observation also corroborated the results of the affinity assays. Monoclonal antibodies DG9 and DG11 failed to exhibit preferential binding to individual virus components in the liquid phase competition assay (Fig. 3), although differences had been observed in the affinity assays. This indicated that deformation of the antigen induced by binding to the trapping antibody influenced recognition by monoclonal antibody. Structural differences induced by antibody binding have been demonstrated in other protein systems (Getzoff et al., 1987). SB2 provided a negative control because no differentiation was evident between top, middle or bottom components in the affinity assay or the liquid phase competition assay.

Unfractionated intact virus (30 μg/ml) incubated with either 1 mg/ml ribonuclease A1 or bovine serum albumin was equally reactive with monoclonal antibodies. Since binding of some
monoclonal antibodies is influenced by denaturation of virus particles with SDS plus urea (Kalmar & Eastwell, 1989), nuclease digestion was also performed on viruses denatured with SDS plus urea (Wu & Bruening, 1971). Nuclease digestion did not alter the binding of monoclonal antibodies to denatured virus. Although these data suggest that the RNA does not participate directly in any of the antigenic determinants recognized by the panel of monoclonal antibodies, it does not preclude the possibility that the protein subunits protect short segments of RNA from nuclease attack near epitopes. It has been previously demonstrated that a few copies of each coat protein subunit VP23 and VP37 bind to RNA after heating in SDS (Daubert & Bruening, 1979).

It is currently believed that there is no processing (i.e. proteolysis) of CPMV or CPSMV capsid proteins specifically associated with encapsidation of RNA (Franssen et al., 1984b). Further modification of intact capsids can occur in the form of proteolytic cleavage of a fragment from the C terminus of the small capsid protein VP23 (Niblett & Semancik, 1969; Geelen et al., 1972; Siler et al., 1976; Kridl & Bruening, 1983), but all three virus components were equally susceptible to this form of processing (Siler et al., 1976). Similar proteolysis of the small capsid protein of CPSMV has been reported (Thongmeearkom & Goodman, 1978). Low level neutron scattering suggested that there were no major differences between the electron density profiles of the protein shells of top, middle and bottom components (Schmidt et al., 1983).
It has been reported that the top component can be resolved from ribonucleoproteins by neutral pH discontinuous PAGE (Siler et al., 1976). This suggested an altered surface charge on virions containing RNA and was the first indication that the capsid structure of the top component differed from that of middle or bottom components.

The present evidence, based on immunological studies, suggests that modification of the capsid surface occurs in response to RNA encapsidation, but RNA does not directly participate in defining the antigenic sites. Although these are novel observations for the comoviruses, similar traits have been demonstrated in related virus systems. Artificially induced empty capsids of picornaviruses (Franssen et al., 1984a) showed definite modification in their antigenic determinants (Meloen et al., 1979; Icenogle et al., 1981; Rombaut et al., 1982). Circular dichroism analysis of the isometric plant virus turnip yellow mosaic virus provided clear evidence that encapsidation of RNA was associated with conformational changes within the protein shell (Tamburro et al., 1978). More extensive analysis of CPMV and CPSMV structure and assembly will be required to ascertain the nature of surface changes induced by the entrapment of viral genomic RNAs.

Since the completion of this work, it has been reported that X-ray crystallography reveals an RNA binding pocket on the inner surface of the large protein subunit (Stauffacher et al., 1989) of beanpod mottle virus, a comovirus closely related to CPSMV (Shepherd, 1963). The proximity of the RNA binding domain to the virion surface suggests that modification of surface structure may occur in response to RNA encapsidation. Indeed, analysis detected differences between crystals of empty virions and those containing RNA.

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REFERENCES

Short communication


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