Influence of the C terminus of the small protein subunit of bean pod mottle virus on the antigenicity of the virus determined using monoclonal antibodies and anti-peptide antiserum

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Middle component particles of bean pod mottle virus (BPMV) containing small protein subunits with a cleaved C terminus were used to produce monoclonal antibodies (MAbs). All MAbs were specific for cryptotopes, i.e. epitopes present only on dissociated BPMV protein. The MAbs reacted more strongly with virus protein preparations containing the cleaved form of the small subunit than with preparations containing only the uncleaved form. It seems that the presence of additional residues at the C terminus of the intact small subunit interferes with antibody binding. Antibodies raised against synthetic peptides corresponding to the C terminus of the uncleaved small subunit reacted with both intact virions and dissociated subunits. This C-terminal region seems to play a dominant role in the antigenicity of the virus.

Introduction

Bean pod mottle virus (BPMV) is a comovirus characterized by the presence of three centrifugal components and contains 60 copies each of a large and a small coat protein subunit (42K and 22K respectively). Each of the three centrifugal components (top, middle and bottom) exists in two electrophoretic forms known as slow migrating and fast migrating (Bancroft, 1962; Thongmeearkom & Goodman, 1978). The slow migrating form of BPMV predominates in the later stages of infection and has a capsid in which a number of C-terminal residues of the small protein subunit have been cleaved off. Since the 13 C-terminal residues of the small subunit are not visible in the three-dimensional structure of BPMV obtained by X-ray crystallography, Chen et al. (1989) have suggested that 13 residues are cleaved off. However, because only trypsin is able to produce the conversion from fast to slow migrating forms (Niblett & Semancik, 1969), it seems likely that the cleavage occurs at arginine 189 and removes only nine residues from the protein. The lower anionic mobility of the cleaved protein is due to the loss of two glutamic acid residues.

There have been a limited number of studies of the antigenic properties of comoviruses (Agrawal & Maat, 1964; Bruening, 1978). Recently, Kalmar & Eastwell (1989a) prepared monoclonal antibodies (MAbs) against cowpea mosaic virus (CPMV) and cowpea severe mosaic virus (CPSMV), and reported that in various immunoassays four of the MAbs bind differentially to the top component of the viruses (Kalmar & Eastwell, 1989b). Of the 15 MAbs raised against the two viruses, six were directed against neotopes (epitopes specific for intact virions), three were directed against cryptotopes (epitopes specific for dissociated viral subunits) and six were directed against metatopes (epitopes recognizing both virions and subunits). In antibody-trapped ELISA, three anti-neotope MAbs reacted better with middle and bottom components than with top component, whereas in a liquid-phase competition assay two of these anti-neotope MAbs could not differentiate the various components. On the other hand the one anti-metatope MAb which differentiated between the centrifugal components reacted better with top component than with the middle and bottom components in antigen-trapped ELISA (Kalmar & Eastwell, 1989b).

In this study we describe the properties of MAbs raised against a preparation of the middle component of BPMV containing only the cleaved form of the small protein subunit. In contrast to the results of Kalmar & Eastwell (1989b), none of the 10 MAbs raised against BPMV was able to detect an antigenic difference between coat protein derived from different centrifugal components of the virus. However, all anti-BPMV MAbs were able to distinguish virions containing only the cleaved form of the small subunit from virions that also contained the uncleaved form. Immunoassays carried out with antibodies prepared against synthetic
peptides corresponding to the C-terminal-cleaved residues of the small subunit demonstrated that the C-terminal region of the subunit played a dominant role in the antigenicity of the virus.

**Methods**

**Viruses and virus proteins.** BPMV (Kentucky G-7 strain) and CPMV (Strain Bi) were obtained from J. E. Johnson (Department of Biological Sciences, Purdue University, West Lafayette, Ind., U.S.A.). BPMV was propagated in *Phasology vulgaris* (cv. Bountiful) and purified by a modified version of the method of Bancroft (1962). Virus concentration was determined by absorbance, using \( A_{260} \) at 1 mg/ml = 8.7. The BPMV centrifugal components were prepared by centrifugation on a cesium chloride gradient (density 1.15 to 1.50) in 0.1 M-phosphate buffer pH 7.0 and concentrated by ultracentrifugation. The concentration of BPMV centrifugal components was calculated from absorbance measurements, using \( A_{260} \) at 1 mg/ml = 8.1 for middle component, \( A_{260} = 10.1 \) for bottom component and \( A_{260} = 1.2 \) for top component (J. E. Johnson, personal communication). Samples of the middle and bottom components of BPMV and CPMV were also obtained from J. E. Johnson. In the case of CPMV, extinction coefficients of \( A_{260} \) at 1 mg/ml of 6.2, 10.0 and 8.1 were used for middle component, bottom component and unfractionated virus respectively. A purified preparation of CPSMV was obtained from C. P. de Jager (Department of Virology, University of Wageningen, The Netherlands).

**Viruses** were propagated in cell cultures of *Vicia faba*, *Phaseolus vulgaris* and *Nicotiana tabacum* var. Samsun NN, and purified by a modified version of the method of Bancroft (1962). Virus concentration was determined by absorbance, using \( A_{260} \) at 1 mg/ml = 8.7. The BPMV centrifugal components were prepared by centrifugation on a cesium chloride gradient (density 1.15 to 1.50) in 0.1 M-phosphate buffer pH 7 for 18 h at 38000 r.p.m. in a 60 Ti rotor (Beckman). Fractions were collected and diluted 10-fold in 0.1 M-phosphate buffer pH 7.0 and concentrated by ultracentrifugation. The concentration of BPMV centrifugal components was calculated from absorbance measurements, using \( A_{260} \) at 1 mg/ml = 8.1 for middle component, \( A_{260} = 10.1 \) for bottom component and \( A_{260} = 1.2 \) for top component (J. E. Johnson, personal communication). Samples of the middle and bottom components of BPMV and CPMV were also obtained from J. E. Johnson. In the case of CPMV, extinction coefficients of \( A_{260} \) at 1 mg/ml of 6.2, 10.0 and 8.1 were used for middle component, bottom component and unfractionated virus respectively. A purified preparation of CPSMV was obtained from C. P. de Jager (Department of Virology, University of Wageningen, The Netherlands).

**Viral protein.** Viral protein was prepared from BPMV top component which had been dialysed at 4 °C for 2 days against 0.1 M-2-(N-cyclohexylamino)-ethanesulfonic acid (CHES) buffer pH 9.9 (J. E. Johnson, personal communication). The dialysate was centrifuged for 50 min at 65000 r.p.m. in a TL 100.2 rotor (Beckman) and the protein was collected from the supernatant.

**Synthetic peptides.** Peptides corresponding to residues 186 to 198 and 180 to 198 (Chen et al., 1989) of the small subunit of BPMV (domain A) were synthesized by the solid-phase method of Merrifield (Barany & Merrifield, 1980).

**Rabbit and chicken antibodies.** Antiseras to BPMV were obtained from rabbits immunized by a series of intramuscular (i.m.) injections of 200 µg antigen (middle component and BPMV protein obtained from J. E. Johnson) in Freund's incomplete adjuvant. Immunoglobulins were prepared by rivanol precipitation using the method of Hardie & Van Regenmortel (1977). Antiseras to peptides were obtained from rabbits immunized by a series of i.m. injections of 100 µg of peptide coupled to ovalbumin by means of glutaraldehyde (Briand et al., 1985). Chicken immunoglobulins specific for BPMV middle component were obtained from yolk derived from eggs of immunized chickens, as described by Polson et al. (1980).

**MAbs.** BALB/c mice (6 weeks old) were immunized according to two protocols: protocol 1 consisted of three intraperitoneal (i.p.) injections (100 µg of purified middle component of BPMV) at 15 day intervals; protocol 2 consisted of only two i.p. injections (50 µg of purified middle component of BPMV). Spleen cells from these mice were used in three cell fusion experiments.

The two mice giving the highest specific antibody response in double antibody-sandwich (DAS)- and in antigen-coated plate (ACP)-ELISA were rested for 1 month and then given two booster injections 4 and 3 days before the fusion experiment (100 µg i.p. 4 days and 10 µg subcutaneously 3 days before the experiment). Three days after the second booster injection, spleen cells were excised and mixed with non-secreting PAI cells (Stocker et al., 1982). Cell fusion was performed using a modified version of the technique described by Fazekas de St Groth & Scheidegger (1980). One fusion experiment (no. 1) was done with mice immunized using protocol 1 and two fusion experiments (no. 2 and no. 3) were done with a mixture of spleen cells from both protocols.

MAbs were obtained in large amounts in ascitic fluids from pristane-primed BALB/c mice which had been injected with 107 hybridoma cells and biotinylated as described by Zrein et al. (1986). Fusion experiment no. 1 gave rise to MAbs 3x1, 19x1 and 23x2, fusion experiment no. 2 to MAbs 8x1, Bx5 and Cx2, and fusion experiment no. 3 to MAbs 1xC4, 2xC4, 3xB4 and 14xA2.

**ELISA.** The procedures have been described in detail elsewhere (Zrein et al., 1986; Dekker et al., 1989).

**Immunosassays used for screening hybridomas.** Supernatants of growing hybridoma cultures were screened for the presence of specific antibodies to BPMV by both ACP- (procedure 1) and DAS- (procedure 2) ELISA using either purified virus or purified coat protein as antigens. Procedure 1 was an antigen-trapped ELISA in which plates were coated overnight at 37 °C with virus or protein at 4 µg/ml in 0.05 M-carbonate buffer pH 9.6. Subsequent steps of the assay were as described by Huguenot et al. (1989).

Procedure 2 was an antibody-trapped ELISA in which the microtiter plates were coated for 3 h at 37 °C with 4 µg/ml of anti-BPMV chicken globulins diluted in carbonate buffer. The subsequent steps were the same as in procedure 1 (Huguenot et al., 1989). A Titertek Multiskan photometer (Flow Laboratories) was used to measure the absorbance at 405 nm. Isotyping of MAbs was done by ELISA procedure 1 using subclass-specific rabbit anti-mouse antiseras (Nordic, ICN) and goat anti-rabbit alkaline phosphatase conjugated antiseras. Purified IgG1, -2a, -2b, -3 and 12 M (Nordic, ICN), and IgA (provided by V. Quesniaux, Basel, Switzerland) were used in control experiments.

**Immunosassays used to analyse the specificity of anti-BPMV MAbs.** Procedure 3 was used to determine serological differentiation index (SDI) values (Jaeglé & Van Regenmortel, 1985). In this test the plates were coated with antigen (3 µg/ml) overnight at 37 °C, biotinylated MAbs in a twofold dilution series in PBS-Tween (T)-bovine serum albumin (BSA) were incubated for 3 h at 37 °C and bound MAbs were detected by the addition of a commercial streptavidin–alkaline phosphatase conjugate (Jackson) diluted 1/4000 in PBS-T–BSA.

In procedure 4, antigen captured by rabbit or chicken antibodies was detected with biotinylated MAbs.

Procedure 5 was an MAAb-trapped ELISA. The MAbs were diluted in a twofold dilution series in coating buffer and incubated for 2 h at 37 °C. The viral antigens (protein or virus) were diluted in PBS-T at a concentration of 1 µg/ml and incubated for 3 h at 37 °C. Rabbit globulins to BPMV or BPMV protein were used to detect captured antigen and were revealed using alkaline phosphatase-labelled goat anti-rabbit globulins (Sigma) diluted 1/2000 in PBS-T.

**Immunosassay used to analyse antiseras to synthetic peptides of BPMV.** Antiseras raised against synthetic peptides were tested for their ability to react with synthetic peptide and purified virus by ACP- (procedure 1) and DAS- (procedure 2) ELISA. In procedure 1, the plates were coated overnight at 37 °C with 5 µM-peptide or 5 µg/ml purified virus. The MAbs were replaced by rabbit antibodies which were revealed using alkaline phosphatase-labelled goat anti-rabbit globulins (Sigma), diluted as in procedure 5. In the DAS-ELISA, virus was used at 2 µg/ml and the first layer consisted of anti-BPMV chicken globulins at 2 µg/ml.

**Gel electrophoresis and immunoblotting.** Virus particles were denatured by 5 min incubation with 2% SDS containing 5% 2-mercaptoethanol. The proteins were analysed by SDS–PAGE on 12-5% polyacrylamide gels (Laemmli, 1970). After electrophoresing onto
**Antigenicity of bean pod mottle virus**

**Fig. 1. SDS–PAGE of different comovirus preparations used for production and characterization of anti-BPMV MAbs.** Staining with Coomassie blue. Lane 1, BPMV middle component containing only the cleaved form of the small subunit used for the production of MAbs (obtained from J. E. Johnson); lane 2, BPMV bottom component containing only the cleaved form of the small subunit (obtained from J. E. Johnson); lane 3, BPMV middle component prepared in Strasbourg and containing the intact and cleaved forms of the small subunit; lane 4, unfractioned CPSMV preparation (obtained from C. P. de Jager) containing the two forms of the small subunit; lane M, Mr standards (phosphorylase b, 94K; bovine serum albumin, 67K; ovalbumin, 43K; carbonic anhydrase, 30K; soybean trypsin inhibitor, 20.1K; α-lactalbumin, 14.4K).

Immobilon polyvinylidenedifluoride membranes (Millipore), viral proteins were incubated in PBS-T containing 2% fatless milk overnight at room temperature. After washing with PBS-T, the Immobilon sheets were incubated with MAb diluted in PBS-T containing 1% fatless milk for 3 h at room temperature. After rinsing with PBS–T, bound MAbs were detected by a 2 h incubation with goat anti-mouse IgG and M conjugated to alkaline phosphatase (Tebu), diluted 1/1000 in PBS-T containing 1% fatless milk. After rinsing, a substrate solution consisting of 50 μg/ml 5-bromo-4-chloro-3-indolyl phosphate, 100 μg/ml nitro blue tetrazolium chloride and 4 mM-MgCl₂ in 0.1 M diethanolamine buffer pH 9.6 was used. A control Immobilon sheet containing electroblotted virus proteins was stained with amido black.

**Results**

**Analysis of virus preparations by SDS–PAGE**

The different virus preparations used in this work were analysed by SDS–PAGE. The results shown in Fig. 1 (lane 1) indicate that the preparation of BPMV middle component used for producing the MAbs and polyclonal antibodies contained only the cleaved form of the small subunit. The preparation of BPMV bottom component provided by Dr J. E. Johnson also contained only the cleaved form of the small subunit (Fig. 1, lane 2). In contrast, the middle component of BPMV purified in our laboratory contained both cleaved and uncleaved forms (Fig. 1, lane 3). It was observed that virus preparations that had been conserved over long periods of time contained decreasing amounts of uncleaved small subunits. When a preparation of another comovirus, CPSMV, containing all three centrifugal components was analysed in the same system, its small subunit also contained both cleaved and uncleaved forms (Fig. 1, lane 4), whereas the preparation of the Bi1 strain of CPMV, which is a bisulphite-generated mutant of CPMV selected for enhanced cleavage and infectivity (Siler et al., 1976), contained only a cleaved form of the small subunit (result not shown).

**Properties of anti-BPMV MAbs**

Clones obtained from the hybridoma fusion experiment were tested both by antigen-trapped and antibody-trapped ELISA. Ten positive clones were obtained which reacted in antigen-trapped ELISA when either BPMV or the virus protein was used as antigen. Not a single positive clone reacting with BPMV or the BPMV protein was detected by antibody-trapped sandwich ELISA (procedures 2 and 4) in which either chicken or rabbit anti-BPMV immunoglobulins were used as the capturing antibody. The capacity of the polyclonal rabbit and chicken antibodies to capture the BPMV protein had been established in control ELISA experiments in which both the capturing antibody on the solid phase and the second antibody were polyclonal antibody preparations. Such a control experiment with chicken antibodies to BPMV is shown in Fig. 2. Since it is known that stable virions such as tobacco mosaic virus (TMV) particles become at least partly dissociated at pH 9-6, leading to preferential adsorption of subunits to the ELISA plate (Dore et al., 1988), it seemed plausible that the reactivity observed with plates coated with BPMV preparations was due to the presence of dissociated viral subunits on the solid phase. When this possibility was tested by adsorbing BPMV particles onto electron microscope grids at pH 9-6 (Dore et al., 1988), it was found that no intact particles were visible. When the microscope grids, which presumably contained BPMV protein, were tested in ELISA as described by Dore et al. (1988), they were found to react with all 10 MAbs. This observation, together with the earlier ELISA results, led to the conclusion that all anti-BPMV MAbs are directed to cryptotopes, i.e. epitopes present only on dissociated viral subunits. Six MAbs were of the μ, κ isotype (Bx5, 19x1, 23x2, 1xC4, 14xA2 and 3xB4), three were of the α,
Fig. 2. Investigation of the capacity of polyclonal chicken antibodies to capture BPMV coat protein using DAS-ELISA (procedure 2). Captured virus subunits were identified using rabbit antiserum to BPMV protein (1/1000) (■), rabbit antiserum to BPMV (1/5000) (■) and mouse antiserum to BPMV (1/5000) (△), followed by anti-immunoglobulin conjugates. Substrate hydrolysis time was 25 min for rabbit and mouse antiserum to BPMV, and 20 min for rabbit antiserum to BPMV protein.

Fig. 3. Investigation of the binding of BPMV coat protein to various MAbs using MAb-trapped ELISA (procedure 5). MAbs 14xA2 (■), 19x1 (□), 3x1 (▲) and 23x2 (▲). Substrate hydrolysis time was 30 min, for MAbs 19x1 and 23x2, and 90 min for MAbs 14xA2 and 3x1.

Fig. 4. Reactivity of MAbs 19x1 (a) and Ax1 (b) with different comovirus preparations in ACP-ELISA (procedure 3). (a) Reactivity of MAb 19x1 with BPMV middle component containing only the cleaved small subunit (■), or both intact and cleaved forms of the small subunit (□). (b) Reactivity of MAb Ax1 with BPMV bottom component (■) containing only the cleaved form of the small subunit and CPMV middle component (○) containing only the cleaved form of the small subunit. Substrate hydrolysis time was 45 min.

Reactivity of the MAbs with the cleaved and intact forms of the small subunit

When used to coat microtitre plates at pH 9-6, all 10 MAbs were able to bind BPMV protein, albeit to different degrees (Fig. 3), but none of the MAbs was able to capture any of the three centrifugal components of BPMV. This result indicated that no epitopes specific for dissociated subunits are accessible in any of the centrifugal components and that our MAbs are unable to differentiate top component from the other two components. When the three centrifugal components were tested by antigen-trapped ELISA, the 10 MAbs reacted in an identical manner with the dissociated subunits derived from the three components.

On the other hand, when preparations of the middle component of BPMV containing either a mixture of cleaved and intact small subunits or cleaved subunits only were compared by antigen-trapped ELISA (procedure 3), all the MAbs were able to differentiate between the two types of preparation (Fig. 4a); the MAbs also differentiated preparations of the top or bottom component of BPMV containing both forms of the small subunit from preparations containing only cleaved subunits. When the ELISA results were plotted as absorbance values against dilutions of ascitic fluid, the
Cross-reactivity of the MAbs with two comoviruses

CPMV and CPSMV were also used to test the reactivity of the anti-BPMV MAbs in ACP-ELISA (procedure 3); CPSMV was used as an unfractionated preparation and CPMV as middle component. The CPSMV preparation contained both the cleaved and intact forms of the small subunit, whereas the CPMV preparation contained only the cleaved form of the small subunit. The ability of MAb Axl to distinguish BPMV containing only the cleaved form of the small subunit from CPMV (strain Bi1), which also contained only cleaved subunits (average 1·7), were larger than those obtained when compared with BPMV containing the two forms of the small subunit. These results suggest that the epitopes recognized by the MAbs differ more between CPMV and BPMV than between CPSMV and BPMV. The SDI values separating BPMV from CPMV and CPSMV are in accordance with earlier serological data which showed that BPMV and CPSMV are more closely related antigenically than are BPMV and CPMV (Agrawal & Maat, 1964; Shepherd, 1963). These results also support the view that CPMV and CPSMV are separate viruses (Swaans & Van Kammen, 1973).

Immunoblotting of BPMV proteins

Immunoblotting experiments were carried out to determine which of the two protein subunits of BPMV was recognized by the various MAbs. A BPMV preparation containing both cleaved and uncleaved forms of the small subunit was subjected to SDS–PAGE and electroblotted onto an Immobilon membrane. None of the MAbs reacted with the cleaved or intact forms of the small subunit, whereas three MAbs of the IgM type (23x2, 3xB4 and 1xC4) reacted with the large subunit. The reaction of MAb 23x2 was stronger than that of the other two MAbs and is illustrated in Fig. 6.

Reactivity of antibodies with C-terminal peptides of the small subunit

The C-terminal sequence of the intact small subunit of BPMV (domain A) is 180AFSVPQANARSEN-198. In the cleaved subunit, residues 190 to 198 (or possibly residues 186 to 198; Chen et al., 1989) have been cleaved off. Peptides corresponding to the sequences 186 to 198 and 180 to 198 were synthesized, coupled to ovalbumin by means of glutaraldehyde and used to raise antibodies in rabbits. The reactivity of the resulting peptide antisera is illustrated in Fig. 7. Both antisera reacted with peptide 180 to 198 adsorbed to the plastic of microtitre plates as well as with BPMV preparations containing both cleaved and uncleaved forms of the small subunit, which were tested by ACP- or DAS-ELISA. These results indicate that the peptide antibodies recognize the C-terminal region of the uncleaved small subunit both in the intact virus particles (DAS-ELISA) and in dissociated protein subunits (ACP-
Fig. 6. Immunoblot of capsid proteins of BPMV containing both intact and cleaved forms of the small subunit. Lane 1, subunits stained with amido black; lane 2, incubation with normal mouse serum (1/500); lane 3, incubation with MAb 23x2 (1/5000). L, large subunit; S, small subunit.

Fig. 7. Reactivity of antiserum to small subunit peptide 180 to 198 (a and b) and to peptide 186 to 198 (c and d) in ACP- (procedure 1, a and c) and DAS-ELISA (procedure 2, b and d) with BPMV containing both intact and cleaved forms of the small subunit (□), or only the cleaved form of the small subunit (■). (a to d) Positive control reaction with peptide 180 to 198 (▲) in ACP-ELISA (procedure 1) and negative control without antigen (△). Substrate hydrolysis time was 15 min (a, b and c) and 30 min (d).

ELISA). As expected none of the peptide antisera reacted with BPMV preparations that contained only the cleaved form of the small subunit.

Discussion

Our results demonstrate that MAbs to comoviruses have the ability to recognize subtle conformational changes in virus coat proteins, as do antibodies to other isometric or rod-shaped plant viruses (Tremaine et al., 1985a, b; Van Regenmortel, 1986; MacKenzie & Tremaine, 1986; Jaegle et al., 1988; Dekker et al., 1989). Such conformational alterations arise for instance when virus subunits polymerize or dissociate, or become adsorbed to plastic during a solid-phase assay. The specificity of our anti-BPMV MAbs is somewhat unusual in that they recognize only the dissociated form of the coat protein despite the fact that the mice used in the fusion experiment had been immunized with intact virus particles. This finding cannot be ascribed to biased screening of hybridoma clones because both BPMV protein (ACP-ELISA) and intact virions (DAS-ELISA) were used as antigen for the screening. Our MAbs were unable to bind to the virus subunits when these had been captured in a sandwich assay by a first layer of polyclonal (rabbit or chicken) antibodies to BPMV or BPMV protein. However, if the MAbs were adsorbed to the solid phase, they were able to capture the virus subunits in solution. The trapped virus protein was then recognized by rabbit antibodies to BPMV or BPMV protein. This difference in reactivity, which depends on the position of the MAbs in the assay, may be explained by assuming that only a small fraction of the antibodies in the polyclonal antiserum is capable of recognizing a surface of the subunit different from that recognized by the MAbs. As a result, the majority of the plate-bound rabbit antibodies used to trap the antigen will not present the subunit in an orientation which allows MAb binding. In contrast, when the subunits are presented by plate-bound MAbs, the small fraction of antibodies in the rabbit serum capable of recognizing the exposed surface of subunits will bind because there is no interference by the other antibodies in the serum.

It is customary to differentiate antibodies raised against a virus coat protein according to their ability to recognize three types of epitopes (Van Regenmortel, 1990): epitopes present only in dissociated virus subunits (cryptotopes), epitopes present only in polymerized subunits in virions (neotopes) or epitopes present in both states of the subunit (metatopes). According to this classification, all the anti-BPMV MAbs are specific for cryptotopes. The capacity of these MAbs to recognize the antigens present in virus or protein preparations in different ELISA formats is shown in Table 1. For comparison, the binding capacity of anti-cryptotope antibodies obtained in the CPMV and TMV systems is also presented in Table 1. The data show that different types of anti-cryptotope antibodies can be distinguished
Table 1. Reactivity in different ELISA formats of anti-cryptotope MAbs obtained in the BPMV, CPMV and TMV systems

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* In this format the virions are dissociated and subunits are preferentially adsorbed to the solid phase (Dore et al., 1988).
† This work.
‡ Kalmar & Eastwell (1989a, b).
§ Dekker et al. (1989).

according to their ability to recognize the virus protein in different immunoassays; for instance, the BPMV MAbs do not bind to virus subunits presented by rabbit antibodies whereas the TMV MAbs do. Another difference is that the BPMV MAbs recognize the subunits adsorbed to plastic whereas the CPMV MAbs do not bind (or bind only very weakly) to subunits presented in this manner.

As expected for anti-cryptotope antibodies, none of the BPMV MAbs is able to differentiate between dissociated virus protein derived from the different centrifugal components of the virus. Kalmar & Eastwell (1989b) have shown that only MAbs to neotopes or metatopes of CPMV and CPSMV exhibit differential binding to centrifugal components, whereas antibodies to cryptotopes are, like our BPMV antibodies, unable to distinguish between protein from the different components.

All the BPMV MAbs reacted better with preparations of virus protein containing cleaved small subunits than with protein preparations also containing the intact form of the small subunit. Therefore it seems that the presence of additional residues at the C terminus of the small subunit interferes with the binding of the MAbs. This phenomenon can be understood by referring to the three-dimensional structure of the BPMV capsid proteins (Chen et al., 1989). Since the crystals used for the X-ray crystallographic analysis of BPMV contained only the cleaved form of the small subunit, the C-terminal residues of the intact form could not be located in the map. However, the C terminus of the cleaved polypeptide is very accessible at the surface of the protein and makes a close contact with the large 42K subunit, in particular with residues 39 to 47 of its B domain. From the location of the cleaved C terminus, it can be inferred that the additional residues in the intact subunit will also have a surface orientation, which could explain why they interfere with the binding of the MAbs.

When the reactivity of the BPMV MAbs was tested by immunoblotting, it was found that three of the antibodies recognized the large protein subunit under denaturing conditions. The other seven antibodies did not react in immunoblotting, presumably because they are specific for conformational epitopes that are destroyed under the conditions of the assay. In the experiments of Kalmar & Eastwell (1989a) some of the MAbs specific for metatopes of CPMV (antibody SB2) and CPSMV (antibody DG4) also reacted in immunoblots with the large subunit of the virus; however, they provided no information on the reactivity of their three anti-cryptotope MAbs in immunoblotting.

Since the presence of the C-terminal residues of the small subunit of BPMV interferes with the binding of MAbs, some of which recognize an epitope on the large subunit (Fig. 6), it must be concluded that in the BPMV protein preparations used in the analysis the small and large subunits remained associated. In the case of BPMV centrifugal components adsorbed directly onto the solid phase at pH 9.6, it is supposed that the proteins are not completely dissociated. Similarly, the method used to prepare BPMV protein (dialysis of top component against CHES buffer pH 9-9) is very mild and unlikely to dissociate subunit aggregates. There is evidence that dissociation of subunits of comoviruses is not complete even in the presence of 5 M-urea and that drastic conditions, such as treatment with SDS and guanidine–lithium chloride, is necessary to obtain the subunits in monomeric form (Wu & Bruening, 1971; Kalmar & Eastwell, 1989a). If the A, B and C domains (Chen et al., 1989) of BPMV protein remain associated, the interference by the C-terminal residues of the small subunit (A domain) on the binding of antibodies to the large subunit is readily explained by the close proximity of the C terminus to the B domain.

It is noteworthy that the antibody (MAb 23x2) which reacts most strongly with the large subunit of BPMV in
immunoblotting (Fig. 6) is the one that is least affected by the presence of the additional C-terminal residues in the intact small subunit (lowest SDI value in Fig. 5). Presumably MAb 23x2 recognizes a continuous epitope in the large subunit which is fairly independent of the steric and conformational effect of the C terminus of the small subunit.

Results obtained in DAS-ELISA with antibodies directed against the C terminus of the intact small subunit confirm that this region is highly accessible both in the protein and in the intact virion, and that it plays a major role in the antigenicity of BPMV. Thus BPMV is similar to many other viruses in which the C terminus of the coat protein also is an immunodominant epitope (see Westhof et al., 1984; Van Regenmortel & Neurath, 1990).

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