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Antigenic Characterization of Potato Virus X with Monoclonal Antibodies

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SUMMARY

A panel of mouse monoclonal antibodies (MAbs) against potato virus X (PVX) was obtained and three of these which had high affinity to the antigen were characterized in detail. These three antibodies defined two epitopes on PVX and recognized native virus, viral coat protein and denatured viral coat protein in various immunological assays. Two of the MAbs and rabbit anti-PVX polyclonal antibodies bound to the 68 amino acid N-terminal peptide of the PVX coat protein. This implies that the N terminus of the PVX coat protein is exposed at the virus surface and forms a highly immunogenic antigenic determinant. In double antibody sandwich (DAS) ELISA, MAbs and their horseradish peroxidase conjugates reacted with PVX at 10 to 20 ng/ml. Monoclonal antibodies to PVX reacted with virus in potato leaves and tubers and detected the virus in DAS ELISA in various combinations, including in combination with polyclonal antibodies.

INTRODUCTION

Potato virus X (PVX) is a potexvirus (Purcifull & Edwardson, 1981) with filamentous particles that contain a M_r 2.1×10^6 single-stranded RNA and M_r 27 000 protein subunits (Tung & Knight, 1972). The sequence of the 237 amino acid PVX coat protein has been deduced from the nucleotide sequence of the cloned 3' terminal part of the PVX genome (Morozov *et al.*, 1983). The strong immunogenic properties of PVX together with its high concentration in infected plants make PVX a suitable model for the immunological analysis of potexviruses.

Polyclonal antibodies (PABs) have been raised to different PVX isolates and successfully used in ELISA for the *in vitro* detection of PVX in potato plants (Banttari & Goodwin, 1985; Bobkova *et al.*, 1983; Goodwin & Banttari, 1984) and in the search for serological relationships between different plant viruses by immunoblotting (Burgermeister & Koenig, 1984).

Monoclonal antibodies (MAbs) have proved useful as tools for the serological differentiation of a number of virus strains and variants which could not be readily distinguished using PABs (Gugerli & Fries, 1983; Halk & De Boer, 1985; Torrance *et al.*, 1986; Massalski & Harrison, 1987; Järvekülg *et al.*, 1987). MAbs have also been used successfully for the antigenic analysis of various plant viruses (Al Moudallal *et al.*, 1982; Tremaine *et al.*, 1985; Koenig & Torrance, 1986; Radavsky *et al.*, 1988). Using rat MAbs to PVX Koenig & Torrance (1986) found three different antigenic determinants on the capsid protein of the B strain of PVX. One determinant was located on, and a second outside, the protruding N terminus of the capsid protein. The third determinant became exposed to MAbs only after some denaturation of the virus particles (Koenig & Torrance, 1986).

In this paper we describe the properties of mouse MAbs raised against a Russian strain of PVX. These MAbs were used for the *in vitro* detection of purified preparations of PVX as well as for the detection of PVX in potato leaves and tubers. PVX capsid protein was cleaved with cyanogen bromide (CNBr) and the reactions of the MAbs with capsid protein fragments were studied by immunoblotting.

METHODS

Viruses. The Russian isolate of PVX (Bobkov *et al.*, 1983) was grown in *Nicotiana tabacum* cv. Xanthi-nc or *Datura stramonium* L. The leaves of infected plants were harvested 3 to 4 weeks after infection and PVX was purified according to Shalla & Shepard (1970). Virus concentrations were estimated spectrophotometrically ($A_{260}^{1\%}$ is 2.9). For tests to detect PVX using ELISA, sap from healthy and infected tubers or leaves was serially diluted in threefold steps in phosphate-buffered saline (PBS) (0.01 M-sodium/potassium phosphate pH 7.4, 0.14 M-NaCl), containing 0.05% Tween 20 and 0.3% bovine serum albumin (BSA) (PBS-TB). The PVX samples included an uncharacterized field isolate of PVX from the Estonian potato cv. Ando and also two 'mild mosaic' and two ring-spot strains of PVX in four Finnish potato cultivars (Kurppa, 1983).

Purified preparations of potato virus M (PVM), potato virus Y (PVY), potato virus S (PVS) and potato leafroll virus used in this study were kindly provided by Professor J. G. Atabekov and Dr V. Novikov (Moscow State University, U.S.S.R.).

PVX coat protein was purified and cleaved with CNBr to give peptides which were isolated as described by Radavsky *et al.* (1988). PVX coat protein was extracted from purified particles by freezing them in 2 M-LiCl for 3 h at -20°C . After thawing, virus RNA was pelleted by centrifugation at 2000 g for 10 min and the soluble coat protein was dialysed against distilled water. For CNBr cleavage, 50 mg of PVX coat protein was dissolved in 2 ml of 85% formic acid. The solution was then diluted with distilled water to 70% formic acid and then mixed with a 70-fold molar excess (per mole of methionine) of CNBr. The mixture was incubated for 24 h at room temperature, diluted eightfold with distilled water and lyophilized. The CNBr fragments were purified by gel filtration on Sephadex G-50, followed by ion-exchange chromatography on DEAE-Toyopearl-650 (Toyo Soda, Japan) as described by Radavsky *et al.* (1988). The amino acid composition and N-terminal sequence of the peptides, including the 68 amino acid N-terminal fragment and the 48 amino acid C-terminal fragment, were analysed by standard methods as described by Radavsky *et al.* (1988).

Production of MAbs. BALB/c mice were injected twice with 100 μg of PVX, first in complete Freund's adjuvant and then in incomplete Freund's adjuvant with 3 weeks between injections. Half of the antigen was administered intraperitoneally and the other half subcutaneously. Four weeks after the second injection the mice were injected intraperitoneally with a booster of 50 μg of antigen in TBS (20 mM-Tris-HCl pH 7.5, 0.15 M-NaCl). Three days later 5×10^7 spleen cells were fused with 3×10^7 non-secreting PAI myeloma cells (Stocker *et al.*, 1982) and hybrids were selected in Iscove's modified Dulbecco's medium (Flow Laboratories) containing 10% foetal calf serum and HAT as described previously (Saarna *et al.*, 1984).

Specific antibodies in the supernatant fluids of the hybrid cultures were assayed after 2 to 3 weeks by the dot immunobinding assay (Hawkes *et al.*, 1982).

Hybridoma cells producing antibodies to PVX were cloned by the limiting dilution method and three of 20 such clones, 21XB4, 21XD2 and 23XA5, were characterized in detail. For mass production of MAbs, approximately 10^7 hybridoma cells in 0.5 ml of TBS were injected intraperitoneally in Pristane-primed BALB/c mice and ascitic fluid was collected from four to six mice 8 to 12 days later, tested for antibody concentration by dot immunobinding, and stored at -40°C .

Antibody purification and enzyme conjugation. Ascites fluids were centrifuged at 3500 r.p.m. for 30 min and the supernatant was mixed with an equal volume of distilled water and two volumes of 20% (w/v) polyethylene glycol 6000 (PEG 6000) (Merck).

The mixture was incubated at 4°C for 30 min, centrifuged at 3500 r.p.m. for 30 min, and the resulting pellet was resuspended in PBS. After a second precipitation with PEG 6000 the antibody solution was dialysed against PBS at 4°C . Electrophoresis in SDS in a 7.5% polyacrylamide gel (Laemmli, 1970) showed that more than 90% of the protein detected was immunoglobulin species. The concentrations of the purified MAbs were 21XB4 18.3 mg/ml, 21XD2 16.5 mg/ml and 23XA5 8.4 mg/ml. Purified MAbs were used throughout this study. Monoclonal antibodies and PABs were conjugated to horseradish peroxidase (HRP) (Olaime, Riga, U.S.S.R.) using the periodate method (Wilson & Nakane, 1978).

Polyclonal antibodies to PVX were isolated from sera from rabbits immunized with purified preparations of PVX. The antisera were produced by injecting rabbits at 3 week intervals with approx. 1 mg purified virus in 1 ml of buffer mixed with an equal volume of Freund's adjuvant. Complete adjuvant was used in the first injection and incomplete in the following injections. Rabbits were immunized by a combination of intravenous and intramuscular injections and were bled at 1 week intervals after two to four injections (Kurppa, 1983). The

immunoglobulin fraction was purified by precipitation with PEG 6000 (Järvekülg *et al.*, 1987), essentially as described above for MAbs.

Indirect ELISA. The wells of the microtitre plates (Flow) were coated with 100 μ l of viral antigen in 50 mM-bicarbonate buffer pH 9.6. The concentration of antigen was from 1.2 to 12 μ g/ml. The coated plates were incubated for 16 h at 4 °C, then washed three times with PBS containing 0.05% Tween 20 (PBS-T). To lower the background binding the wells were blocked with 200 μ l of PBS-T containing 1% BSA, for 2 h at room temperature. The plates were washed by rinsing three times with PBS-T and then antibodies diluted in 100 μ l of PBS-TB were pipetted into the wells. The plates containing antibodies were incubated at 37 °C for 2 h and then washed five times with PBS-T. A 1:1000 dilution of rabbit anti-mouse IgG-HRP conjugate or goat anti-rabbit IgG-HRP conjugate (Sigma) in 100 μ l of PBS-TB was then added. The plates were incubated at 37 °C for 1 to 2 h and then washed five times with PBS-T. Next, 100 μ l of substrate solution [2,2'-azino-di(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS; Boehringer Mannheim) in 0.05 M-citrate-phosphate buffer pH 4.0, containing 0.01% H₂O₂ and/or *o*-phenylenediamine (OPD) in a 0.1 M-citrate-phosphate buffer pH 5.0, containing 0.01% H₂O₂] was added to each well. Absorbances were measured at identical time intervals (from 15 to 30 min) at 414 nm (ABTS) or 450 nm (OPD) using a Titertek Multiskan MC photometer.

Double antibody sandwich (DAS) ELISA. DAS ELISA (Clark & Adams, 1977) was performed using MAbs or PAbs as coating antibody and HRP-conjugated MAbs as second antibody. The wells of the microtitre plates were coated with 100 μ l of 0.05 M-bicarbonate buffer pH 9.6 containing MAbs (1 to 20 μ g/ml) or PAbs (2.5 to 5 μ g/ml). The microtitre plates were incubated at 4 °C for 16 h. After blocking and washing the wells as for indirect ELISA, the antigen in PBS-TB was incubated in the wells for 2 h at 37 °C. Purified PVX was serially diluted in PBS-TB to between 6 ng/ml and 6 μ g/ml. Plant samples were prepared by diluting the sap of leaves or potato tubers 10-fold in PBS-T and then making a threefold dilution series in PBS-TB. After incubation with the antigen, the wells were washed five times with PBS-T and a 1:500 to 1:2000 dilution of HRP-conjugated anti-PVX MAb in 100 μ l of PBS-TB was added to each well. The concentration of conjugate was from 1 to 5 μ g/ml. After incubation of the plates for 2 h at 37 °C and five washes with PBS-T, 100 μ l of substrate solution was added. The plates were incubated for 15 to 30 min, and reactions were determined spectrophotometrically with a Titertek Multiskan MC.

Immunoblotting. SDS-PAGE was in a discontinuous buffer system (Laemmli, 1970) in 15% acrylamide containing 4 M-urea. After electrophoresis, proteins were transferred to BA85 nitrocellulose paper (Schleicher & Schüll) essentially as described by Towbin *et al.* (1979). After transfer (5 V/cm, 1 to 4 h) one part of the nitrocellulose sheet was washed five times with TBS, blocked with 10% donor calf serum in TBS at 37 °C for 2 h, and washed five times with TBS. Then the nitrocellulose blot was reacted with anti-PVX MAbs or PAbs which had been diluted 1:1000 in TBS containing 10% donor calf serum. After five washes with TBS the nitrocellulose was reacted with HRP-conjugated rabbit anti-mouse IgG in 10% donor calf serum with TBS for 2 h at room temperature. The secondary antibody dilution was 1:100. The filter was washed five times with TBS and the colour reaction was developed with 0.25 mg/ml 4-chloro-1-naphthol (Sigma), 0.03% H₂O₂ in TBS for 10 to 20 min at room temperature in the dark. The second part of the nitrocellulose blot was stained with amido black.

Determination of antibody isotypes. The IgG isotypes of the MAbs were determined by double antibody enzyme immunoassay using a mouse hybridoma sub-isotyping kit (Calbiochem). The subcloned hybridoma supernatant fluids were tested according to the manufacturer's instructions.

Competition ELISA. Purified MAb 21XD2 was conjugated to HRP using the periodate method (Wilson & Nakane, 1978). The wells of the microtitre plates were coated with 100 μ l of PVX or PVX capsid protein (0.1 μ g/ml) in TBS for 12 h at 4 °C and blocked with 1% BSA in TBS for 2 h at room temperature. Serial dilutions of unlabelled antibodies (0.01 to 100 μ g/ml) and non-saturating constant amounts of HRP-labelled antibodies (10 ng/ml) were mixed to compete in various combinations in these wells. The reaction was carried out for 12 h at 4 °C in TBS containing 1% BSA and 0.05% Tween 20. Readings were taken with a Titertek Multiskan MC reader with OPD as the substrate essentially as described above.

RESULTS

A panel of hybridoma cell lines was selected which secreted MAbs to PVX. From 20 different hybridoma cell lines, three were selected for further work. The MAbs 21XB4, 21XD2 and 23XA5 secreted by these cell lines were characterized in detail in reactions with PVX in infected plant material, with purified virus preparations, with viral coat protein and with coat protein peptide fragments.

Characterization of monoclonal antibodies

Mouse anti-PVX MAbs 21XB4, 21XD2 and 23XA5 were first tested in indirect ELISA with purified PVX. At antigen concentrations of 1.2 μ g/ml, 6 μ g/ml and 12 μ g/ml, all three MAbs had high titres (Fig. 1) and were specific for PVX in that the ELISA readings at $A_{414 \text{ nm}}$ with other

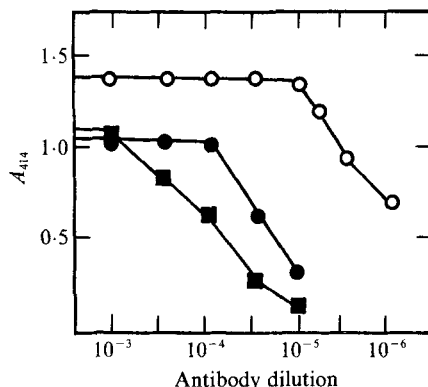


Fig. 1. Comparison of reactivity of MABs 21XB4 (○), 21XD2 (●) and 23XA5 (■) in indirect ELISA using PVX-coated (12 µg/ml) immunoplates. Purified MAB concentrations were MAB 21XB4, 18.3 mg/ml; MAB 21XD2, 16.5 mg/ml; MAB 23XA5, 18.4 mg/ml. Substrate was ABTS.

viral antigens at 1 µg/ml were PVM 0.05, PVS 0.042, PVY 0.022, and tobacco mosaic virus 0.06 i.e. at least two orders of magnitude lower than comparable readings for PVX. It is evident from Fig. 1 that MABs 21XD2 and 23XA5 have titres in ELISA of about 1:10⁵, but that of MAB 21XB4 is about 1:10⁶. All three anti-PVX MABs also recognized PVX in plant samples (see below). From the ELISA titration curves (Fig. 1) we calculated the apparent dissociation constants (K_d) for these MAB-antigen complexes according to the Scatchard equation (Friquet *et al.*, 1985) to be for MAB 21XB4 1.5×10^{-10} M, for 21XD2 1.2×10^{-9} M and for 23XA5 1.9×10^{-9} M. MABs 21XB4, 21XD2 and 23XA5 also reacted with ¹²⁵I-labelled Protein A (Amersham); MABs 21XD2 and 23XA5 were shown to be IgG2b, whereas MAB 21XB4 was of subclass IgG2a.

Epitope analysis

Epitope specificity of the different MABs was determined by competition ELISA on PVX and PVX capsid protein adsorbed to plates. Positive competition in antibody binding was based on the assumption that if two antigenic sites were close or overlapping then the binding of an antibody to one of the sites would hinder the binding of other antibodies to it and the other site. In order to minimize the effect of the different binding constants of MABs and to favour the inhibition reaction, different dilutions of unlabelled antibody were incubated overnight before adding HRP-conjugated MAB 21XD2. MABs 21XD2 and 23XA5 competed with, and suppressed reaction by the MAB 21XD2-HRP conjugate completely, but MAB 21XB4 had no effect on the reaction of MAB 21XD2. Thus MAB 21XB4 defines one independent PVX epitope and MABs 21XD2 and 23XA5 define a second independent PVX epitope.

Thus, these three MABs to PVX react with at least two independent antigenic regions on PVX. MAB 21XB4 binds with high affinity to one antigenic site and MABs 21XD2 and 23XA5 with lower affinity to one or two close antigenic sites. Despite slight differences between the binding constants of MABs 21XD2 and 23XA5 it remains possible that they recognize the same epitope of PVX.

Interaction of monoclonal antibodies with PVX, PVX coat protein and CNBr cleavage fragments of the coat protein

To determine whether any of the MABs reacted with denatured viral coat protein, we assayed them by immunoblotting (Towbin *et al.*, 1979). The results show that MABs 21XD2 and 23XA5 react with denatured viral coat protein (Fig. 2a, b) and that MAB 21XB4 does not. However, at an IgG concentration of 100 µg/ml, MAB 21XB4 reacted non-specifically with denatured PVX capsid protein because it also reacted with the molecular weight marker proteins (data not shown). In contrast the immunoblot of PVX-infected potato leaf sap with MABs 21XD2 and

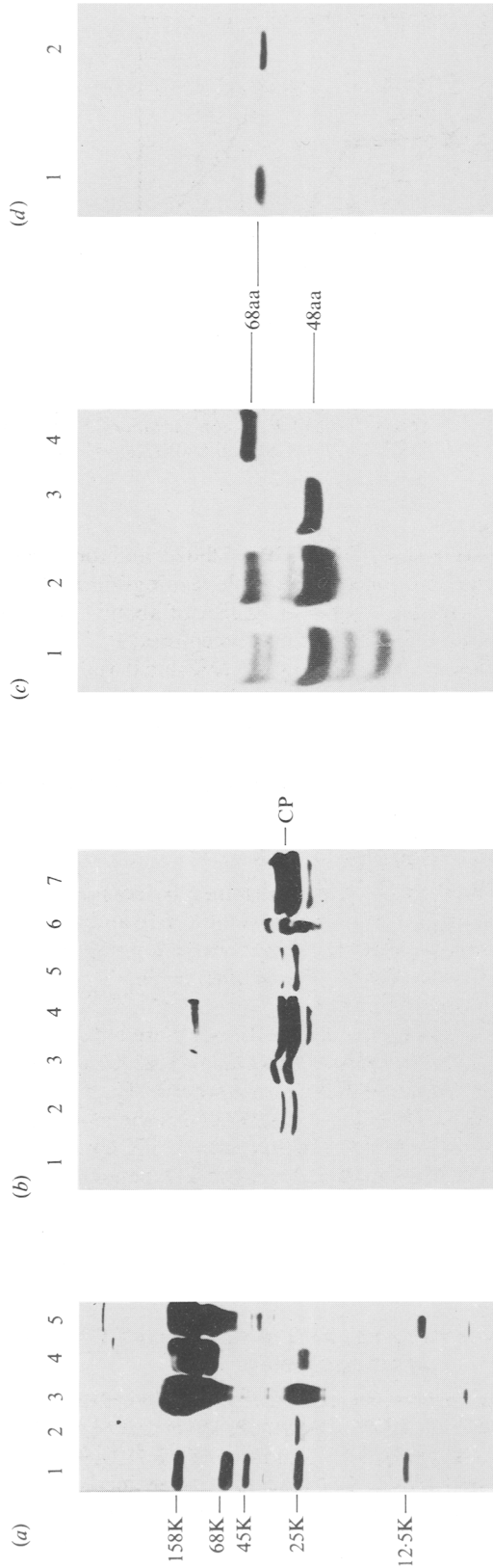


Fig. 2. SDS-polyacrylamide gel electrophoresis and immunoblotting of PVX coat protein and CNBr peptides. Electrophoresis was in 12% (a, b) or 10 to 30% gradient gels (c, d). (a) Gel stained with Coomassie Brilliant Blue. Samples were (lane 1) M_r standards aldolase (158K), BSA (68K), ovalbumin (45K), chymotrypsinogen A (25K) and cytochrome c (12.5K), (lane 2) PVX (2 μ g), (lanes 3 and 4) extract of PVX-infected potato leaf extract diluted 1/10 (lane 3) and 1/25 (lane 4) and (lane 5) healthy potato leaf extract diluted 1/10. (b) Immunoblot of PVX (lanes 1, 4 and 7; 2 μ g) and PVX-infected potato leaf extract diluted 25-fold (lanes 2 and 5) and 10-fold (lanes 3 and 6) reacted with MAb 21XB4 (lane 1), 21XD2 (lanes 2, 3 and 4) and 23XA5 (lanes 5, 6 and 7). CP, PVX coat protein. (c) Gel stained with Coomassie Brilliant Blue. PVX coat protein was cleaved with CNBr and the fragments were separated by SDS-PAGE in a 10 to 30% gradient gel. Samples were an unfractionated mixture of PVX coat protein CNBr cleavage fragments (lane 1), 3 μ g of partially purified PVX coat protein CNBr cleavage mixture (lane 2), 3 μ g of the 48 amino acid (aa) PVX coat protein C-terminal fragment (lane 3) and 1 μ g 68 aa PVX coat protein N-terminal fragment (lane 4). (d) Immunoblot of unfractionated CNBr fragments of PVX coat protein with MAbs 21XD2 (lane 1) and 23XA5 (lane 2).

23XA5 showed a strong reaction with PVX coat protein but no anomalous staining of plant proteins (Fig. 2*b*).

The primary structure of the PVX coat protein has recently been deduced from the sequence of the 708 nucleotide coat protein gene (Morozov *et al.*, 1983). This permits calculation of the sizes of the CNBr cleavage fragments of the coat protein and the determination of the location of epitopes to these peptides using MAbs.

After cleavage with CNBr the protein fragments were separated by electrophoresis in a 10 to 30% SDS-PAGE gradient gel. At least six fragments were visible after staining with Coomassie Brilliant Blue (Fig. 2*c*, lane 1). The N-terminal amino acids Leu, Ile, Ala, Asp, Lys and Pro were identified in the CNBr cleavage mixture of PVX capsid protein; these correspond to the correct and complete cleavage at Met-X sites of the protein (Radavsky *et al.*, 1988). The mixture of coat protein fragments was fractionated on Sephadex G-50 and of the five fractions separated the first one only (Fig. 2*c*, lane 2) showed immunoreactivity with MAbs 21XD2 and 23XA5 (Radavsky *et al.*, 1988). This fraction contained three peptide fragments (Fig. 2*c*, lane 2) and was further purified by means of ion-exchange chromatography on DEAE-Toyopearl-650 (Radavsky *et al.*, 1988). Of two separated homogeneous fractions (Fig. 2*c*, lanes 3 and 4), only one which contained the larger CNBr fragment reacted with MAbs 21XD2 and 23XA5 in an indirect ELISA. This fragment (Fig. 2*c*, lane 4) is the 68 amino acid N-terminal fragment and the shorter one is the 48 amino acid C-terminal fragment (Fig. 2*c*, lane 3) (Radavsky *et al.*, 1988). The location of antigenic determinants was further analysed by immunoblotting the mixture of CNBr fragments and purified peptides with MAbs 21XD2, 21XB4 and 23XA5. The blotting data clearly show that the larger CNBr fragment of the PVX coat protein reacts with MAbs 21XD2 and 23XA5 (Fig. 2*d*, lanes 1 and 2). We conclude that the antigenic determinants for MAbs 21XD2 and 23XA5 are located at the N terminus of the coat protein. MAb 21XB4 was not reactive with the capsid protein CNBr fragments. Anti-PVX polyclonal antibodies also reacted with the N-terminal fragment of the PVX coat protein (Radavsky *et al.*, 1988).

Identification of PVX in plant samples

The results of the DAS ELISA with different concentrations of purified PVX (Fig. 3) show that homologous and heterologous MAb combinations readily detected PVX at concentrations as low as 10 to 20 ng/ml with low background values and that the best antibody for coating was 23XA5 and conjugate 21XD2-HRP was slightly better than conjugate 21XB4-HRP.

Other tests were done using sap from PVX-infected potato leaves and tubers. The virus was detected in extracts from infected tissues at high dilutions (Fig. 4), whereas similarly treated extracts from healthy plants gave no detectable reaction. As with purified PVX preparations, MAb 23XA5 was the best coating antibody. MAb 21XB4, which had the highest apparent dissociation constant (K_d 1.5×10^{-10} M), gave satisfactory results, applied as either the first or second antibody (Fig. 4).

Indirect ELISAs and DAS ELISAs were done to compare the interaction of MAbs with PVX isolates. MAbs 21XB4, 21XD2 and 23XA5 recognized common strains ('mild mosaic' strains) and ringspot strains of Finnish PVX isolates (listed in the paper by Kurppa, 1983) and also all PVX isolates available in the U.S.S.R up to now. Therefore, MAbs 21XD2, 21XB4 and 23XA5 seem to be specific to epitopes common to several PVX isolates.

DISCUSSION

Potato virus X is distributed world-wide and has been studied with PAbs (Rich, 1983) and with MAbs (Torrance *et al.*, 1986; Koenig & Torrance, 1986; Järvekülg *et al.*, 1987; Radavsky *et al.*, 1988). The aim of this study was to obtain MAbs to PVX that would be suitable for routine serological identification of PVX and to localize viral epitopes. We have described the production and testing of three mouse MAbs specific for PVX. In the indirect ELISA test MAbs 21XB4, 21XD2 and 23XA5 reacted with native PVX particles, showing apparent dissociation constants of 1.5×10^{-10} M, 1.2×10^{-9} M and 1.9×10^{-9} M, respectively, and did not react with other potato viruses. MAbs 21XD2 and 23XA5 reacted with the native and denatured coat protein of PVX (Fig. 2*b*), but MAb 21XB4 recognized only the non-denatured coat protein. This

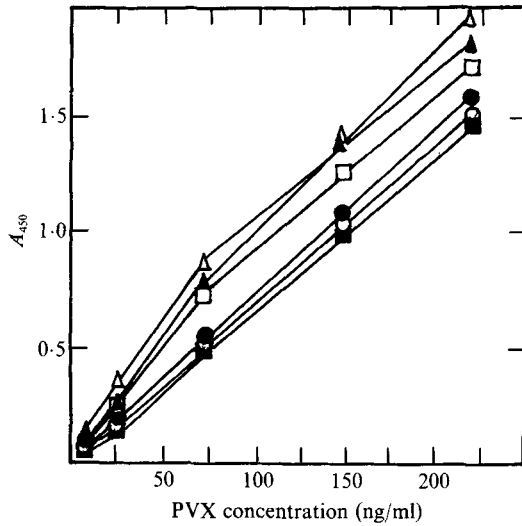


Fig. 3. Detection of purified preparations of PVX in DAS ELISA with MAbs. The combinations were coating with 21XB4 and detection with HRP-conjugated 21XB4 (○) or 21XD2 (●), coating with 23XA5 and detection with conjugated 21XB4 (△) or 21XD2 (▲), and coating with 21XD2 and detection with conjugated 21XB4 (□) or 21XD2 (■).

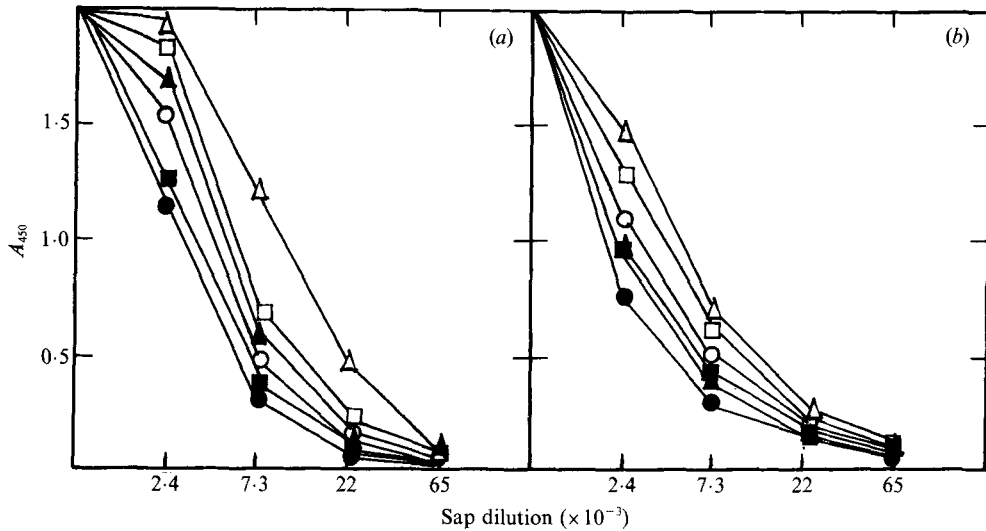


Fig. 4. Detection of PVX by DAS ELISA in potato leaf extracts (a) and potato tuber extracts (b). The combinations were coating with 21XB4 and detection with HRP-conjugated 21XB4 (○) or 21XD2 (●), coating with 23XA5 and detection with 21XB4 (△) or 21XD2 (▲), and coating with 21XD2 and detection with 21XB4 (□) or 21XD2 (■).

indicated that MAbs 21XD2 and 23XA5 are specific for linear epitopes of PVX coat protein, but MAb 21XB4 seems to recognize a topographic or conformation-dependent epitope. Moreover, MAbs 21XD2 and 23XA5 recognized the 68 amino acid N-terminal CNBr fragment of PVX capsid protein in immunoblotting (Fig. 2d). This implies that these three MAbs define at least two epitopes on PVX, a conclusion supported by the results of competition ELISA. MAbs 21XD2 and 23XA5 may bind either to the same linear epitope or to closely adjacent or

overlapping epitopes. However, we conclude that at least one PVX epitope is located in residues 1 to 68 of the virus coat protein which is antigenic also in the assembled virus particle, i.e. is located on the PVX particle surface. The C terminus of the PVX capsid protein also seems to be located on the virus surface, since PABs react with the 48 amino acid C-terminal CNBr fragment (Radavsky *et al.*, 1988). Our results are in accord with those of Koenig *et al.* (1978), which showed that N- and C-terminal portions of the capsid protein can be split off with proteases and that the resulting PVX particles can be distinguished by their reactions with PABs (Koenig, 1972, 1978). Using rat MAbs to PVX, Koenig & Torrance (1986) clearly distinguished PVX particles with capsid proteins lacking N or C termini and found three antigenic determinants on the protein subunits of the B strain of PVX. Their MAbs of group II were specific for the protruding N terminus of the PVX capsid protein and in this respect are similar to our MAbs 21XD2 and 23XA5. However, unlike MAbs 21XD2 and 23XA5, which bind to a linear epitope in the denatured PVX capsid protein N terminus, the group II MAbs of Koenig & Torrance (1986) reacted extremely weakly with SDS-denatured virus. Although the exact location of the epitope recognized by group II MAbs was not determined, MAbs of groups I and III (Koenig & Torrance, 1986) were specific for determinants outside the protruding N terminus. The determinants reacting with MAbs of group I were destroyed by SDS, in contrast to those of group III, which recognized denatured capsid protein lacking the N- and the C-terminal portions. MAbs 21XD2 and 23XA5 thus resemble those of group III in reacting with the denatured capsid proteins. Unfortunately, direct comparison of the epitopes recognized by these MAbs is not possible since the exact positions of N-terminal and C-terminal cleavage of the capsid protein were not determined (Koenig *et al.*, 1978; Koenig & Torrance, 1986).

The initial aim of this study, that of producing MAbs for routine DAS ELISA identification of PVX, has been achieved (Fig. 3, 4). In DAS ELISA our MAbs to PVX and their HRP conjugates reliably detected PVX at 10 to 20 ng/ml (Fig. 3). PVX was detectable with DAS ELISA in sap from leaves or tubers of field samples of potato diluted up to 10000-fold (Fig. 4). For plant virus detection, the limits of sensitivity for direct or indirect ELISA with PABs were reported to be around 1 ng/ml (Clark & Adams, 1977; Clark *et al.*, 1986). However, in our hands PABs and their HRP conjugates gave the same sensitivity in DAS ELISA as MAbs. Additionally, in DAS ELISA MAbs to PVX work equally well in various combinations with PABs.

Serological identification of viruses is possible with antibody mixtures having broad specificities or with antibodies specific to a single, but common, antigenic determinant. Monoclonal antibodies 21XB4, 21XD2 and 23XA5 recognize several strains of PVX and thus seem to be specific for an epitope common to these isolates. This proposal needs further investigation, since the number of PVX isolates tested was small compared to the large number of isolates tested by Torrance *et al.* (1986). In contrast to our results Torrance *et al.* (1986) described MAbs to PVX that allow the serological classification of PVX. In terms of serotype specificity the results of our group and those of Torrance *et al.* (1986) are not directly comparable. To overcome this problem, a direct experimental comparison is necessary.

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