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## Antigenic Analysis of Potato Virus X by Means of Monoclonal Antibodies

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### SUMMARY

At least three different antigenic determinants were distinguished on the capsid protein of the B strain of potato virus X by their differential reactivity with monoclonal antibodies. One determinant (or group of determinants) was located on the protruding N terminus which, in the assembled virus particles, is readily split off by proteases in crude plant sap or by trypsin. A second determinant (or group of determinants) was located outside the protruding N terminus on the surface of the undisturbed virus particles. In partially denatured preparations containing the protruding N terminus, this determinant became inaccessible. A third determinant (or group of determinants) became exposed only after some denaturation of the virus particles, e.g. when they were applied directly to ELISA plates or nitrocellulose membranes. In contrast to the other two determinants, this determinant was not destroyed by extensive denaturation, such as heating in solution with SDS and 2-mercaptoethanol.

### INTRODUCTION

Monoclonal antibodies have proved to be powerful tools for the antigenic analysis of many proteins and proteinaceous structures including plant viruses (e.g. Al Moudallal *et al.*, 1982; Dougherty *et al.*, 1985; Tremaine *et al.*, 1985). Potato virus X (PVX) would seem to be especially suitable for such studies, because N- and C-terminal portions of the capsid protein can be split off in the assembled virus particles by treatment with reducing agent-dependent proteases in crude plant sap or trypsin, and with reducing agent-independent proteases in some partially purified virus preparations, respectively (Koenig *et al.*, 1978). Preparations of virus particles with either intact or partially degraded N or C termini can only be distinguished with difficulty by their reactions with polyclonal antisera (Koenig, 1972, 1978). With the monoclonal antibodies prepared by Torrance *et al.* (1986), we were able to distinguish such preparations and to define at least three antigenic determinants on the protein subunits of the B strain of PVX when native and denatured virus preparations were studied in various serological tests.

### METHODS

*Virus isolates and preparations.* Three isolates of PVX were used in this study: the CsAg and M strains (Koenig *et al.*, 1978) and the B strain (Matthews, 1948; Shepard & Shalla, 1972). Virus preparations were those from earlier studies (Koenig *et al.*, 1978; R. Koenig, unpublished) that had been kept frozen in 10 mM-sodium citrate pH 7.0 containing 20% glycerol. Their behaviour in SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was rechecked before the experiments were started (Fig. 1).

*Preparations of monoclonal antibodies (MAbs).* MAbs were prepared as described by Torrance *et al.* (1986) by fusing spleen cells from rats immunized against PVX with one of two rat myeloma lines, Y3 Ag1.2.3 (Galfrè *et al.*, 1979) or YB2/3.OAg20 (Kilmartin *et al.*, 1982). The cells secreting MAbs 58, 59 and 66 originated from an anti-PVX strain DX fusion and MAbs 68 to 73 from an anti-PVX strain HB fusion. The methods for cell fusion and maintenance were as described by Galfrè & Milstein (1981). Supernatant fluids were obtained from growing cultures.

*Testing procedures.* For dot-blot immunoassays, aliquots of virus preparations with precipitin titres of at least 1:1024 were diluted 1:20 in the following solutions: (a) 10 mM-Tris-HCl pH 7.4, (b) 200 mM-Tris-HCl pH 8.2 containing 0.4% trypsin, (c) 10 mM-Tris-HCl pH 7.4 containing 5% pyrrolidine, (d) 2% SDS, 5% 2-mercaptoethanol, 10% glycerol, 0.001% bromophenol blue, 125 mM-Tris-HCl pH 6.8 (Laemmli & Favre, 1973). After dilution in buffer (b) the virus was incubated for 1 h at 37 °C, after dilution in buffer (d) the preparations were placed in a boiling water-bath for 3 min. Dots of 1 µl were placed onto the nitrocellulose membranes. The free protein-binding sites of the membranes were then blocked by treatment for at least 2 h with a solution containing 1% bovine serum albumin, 10 mM-Tris-HCl pH 7.4, 0.85% NaCl. Tissue culture supernatants containing MAbs were diluted 1:10 in the same solution, supplemented with 0.05% Tween 20, 0.3% Triton X-100, 0.05% sodium azide. After overnight incubation with the MAb-containing solutions, the membranes were washed seven times with 0.85% NaCl and the binding of the MAbs was then detected by reaction with alkaline phosphatase-labelled goat anti-rat antibodies following the procedure described by Burgermeister & Koenig (1984), but using the colour reaction recommended by Bode *et al.* (1984).

SDS-PAGE (Laemmli & Favre, 1973), electro-blot immunoassay (Burgermeister & Koenig, 1984), drop precipitin tests (Bercks *et al.*, 1972) and indirect ELISA on plates which were or were not precoated with polyclonal antibodies (Koenig, 1981) were done as in the published descriptions. Tissue culture supernatants containing MAbs were used undiluted in the drop precipitin test and at a dilution of 1:10 in ELISA and the electro-blot immunoassay.

## RESULTS

Fig. 1 shows the behaviour in SDS-polyacrylamide of different preparations of PVX used in this study. The four types of subunit which may be found in PVX preparations (Koenig *et al.*, 1978) are seen in lane 1. The most slowly moving band is the intact protein PVX-P<sub>s</sub>, followed by PVX-P<sub>f</sub> which lacks a C-terminal portion, PVX-P<sub>r</sub> which lacks a N-terminal portion and PVX-P<sub>o</sub> which lacks both the N- and the C-terminal portions due to the action of plant proteases. PVX-P<sub>td</sub>, which is obtained after treatment of virus preparations containing PVX-P<sub>s</sub> with trypsin and which also lacks an N-terminal portion, is seen in lanes 3, 7 and 11. It moves somewhat more slowly than PVX-P<sub>f</sub> shown in lanes 4, 8 and 12.

The MAbs described by Torrance *et al.* (1986) could be divided into three groups on the basis of the reaction patterns they gave with differently treated preparations of the B strain of PVX in two indirect ELISA procedures, the drop precipitin test and dot-blot immunoassay (Table 1). Essentially the same results as obtained with the B strain were also obtained with differently treated preparations of the CsAg and M strains, except that these two strains failed to react with the MAbs in group II and with MAb 71 in group III. None of the three strains reacted with MAb 67 which has been found to be specific for two South American isolates (Torrance *et al.*, 1986). In indirect ELISA on plates precoated with polyclonal antibodies the MAbs of group I reacted with virus preparations regardless of whether they contained PVX-P<sub>s</sub> or PVX-P<sub>f</sub> (Table 1). The MAbs of group II, however, reacted in this test only with preparations containing PVX-P<sub>s</sub> and those of group III did not react at all. However, when the virus particles were attached directly to the plates, group III MAbs did react, regardless of whether the preparations contained PVX-P<sub>s</sub> or PVX-P<sub>f</sub>, but the reactivity of the MAbs of groups I and II with preparations containing PVX-P<sub>s</sub> was lost. Surprisingly, the reactivity of the group I MAbs with preparations containing PVX-P<sub>f</sub> was retained under these conditions. These phenomena were observed regardless of whether the virus was applied to the plates in coating buffer (50 mM-sodium carbonate, pH 9.6) or phosphate-buffered saline, pH 7.3 (Clark & Adams, 1977).

Drop precipitin tests done with undiluted MAbs gave similar results to indirect ELISA on plates precoated with polyclonal antibodies. The failure of two MAbs in group II to react might have been due to a lower antibody content.

The results of dot-blot immunoassays in which the virus was applied directly to the nitrocellulose membranes were in some respects intermediate between those of the two indirect ELISA procedures. Untreated virus preparations were detected by the MAbs of group I, as well as by those of group III, regardless of whether the virus contained PVX-P<sub>s</sub> or PVX-P<sub>f</sub>. Group II MAbs, however, again detected only preparations containing PVX-P<sub>s</sub>. Removal of the protruding N terminus from these preparations by trypsin treatment destroyed their reactivity with this group of MAbs, but not with those of groups I and III.

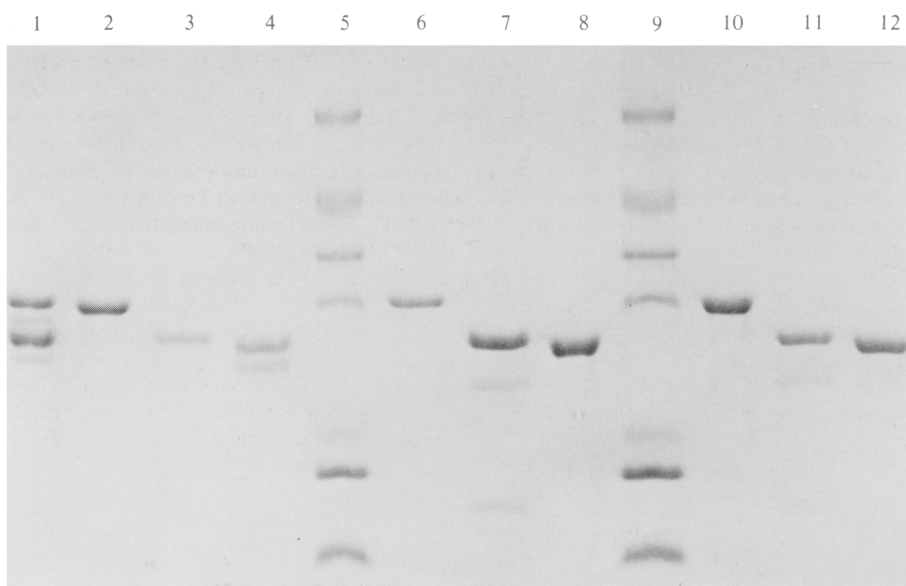


Fig. 1. SDS-PAGE of the protein subunits of preparations of the B (lanes 1 to 4), CsAg (lanes 6 to 8) and M (lanes 10 to 12) strains of PVX. Lane 1 contains (from top) PVX-P<sub>s</sub>, PVX-P<sub>i</sub>, PVX-P<sub>f</sub> and PVX-P<sub>u</sub>. Lanes 2, 6 and 10 contain PVX-P<sub>s</sub>, lanes 3, 7 and 11 contain PVX-P<sub>id</sub> and lanes 4, 8 and 12 contain PVX-P<sub>f</sub>. Lanes 7 and 11 also contain some smaller tryptic peptides and lane 4 also contains PVX-P<sub>u</sub>. For the meaning of the abbreviations see text. Lanes 5 and 9 contain as marker proteins, bovine serum albumin (mol. wt. 66000, 66K), ovalbumin (45K), glyceraldehyde-3-phosphate dehydrogenase (36K), carbonic anhydrase (29K), coat protein subunits of turnip yellow (20K) and tobacco mosaic (17.8K) viruses and lysozyme (14.3K).

Treatment of the virus preparations with 5% pyrrolidine or 2% SDS and 5% 2-mercaptoethanol destroyed their reactivity with the MAbs of group I, but not with those of group III. The reactivity of group II MAbs with SDS-degraded virus was extremely weak, but a reactivity with pyrrolidine-degraded virus was clearly detectable.

In electro-blot immunoassays, only the MAbs of group III were reactive, which is understandable in view of the lability in SDS of the determinant's reaction with the MAbs of groups I and II. Group III MAbs reacted with PVX-P<sub>s</sub>, PVX-P<sub>i</sub>, PVX-P<sub>f</sub>, PVX-P<sub>u</sub> and PVX-P<sub>id</sub> of the B strain of PVX. The reactivity of MAb 73 with intact PVX protein and proteins lacking C and/or N terminal portions is shown in Fig. 2.

#### DISCUSSION

A schematic interpretation of our results is given in Fig. 3. The MAbs of group II are obviously specific for the protruding N terminus of the capsid protein of the B strain of PVX, because in indirect ELISA on plates precoated with polyclonal antibodies and in dot-blot immunoassay, they reacted only with preparations containing PVX-P<sub>s</sub>, but not with those containing PVX-P<sub>f</sub> or PVX-P<sub>id</sub>.

The MAbs of groups I and III, on the other hand, seem to be specific for determinants outside the protruding N terminus, because they reacted with virus preparations regardless of whether these contained PVX-P<sub>s</sub>, PVX-P<sub>f</sub> or PVX-P<sub>id</sub>. The two (groups of) determinants reacting with the MAbs of groups I and III, respectively, can be clearly distinguished by their differential reactivity in various serological tests (Table 1). Differences in the reactivity of MAbs in various ELISA procedures have also been observed by others, e.g. Al Moudallal *et al.* (1984). Jaegle & van Regenmortel (1985) have suggested that virus particles may become partially denatured when they are applied directly to ELISA plates. Our results seem to confirm this. The MAbs of group III are apparently specific for determinants which become exposed in particles of the B,

Table 1. Reactivity of three groups of MAbs in different serological tests with differently treated preparations of the B strain\* of PVX which contained either intact subunits (PVX-P<sub>s</sub>) or subunits (PVX-P<sub>f</sub>) lacking an N terminal portion

Testing procedure†	Treatment of virus preparation	Group of MAbs (code number of individual clones in parentheses) and type of subunit in untreated virus preparation					
		I (58, 59, 66)		II (68, 69, 70)		III (71, 72, 73)	
		PVX-P <sub>s</sub>	PVX-P <sub>f</sub>	PVX-P <sub>s</sub>	PVX-P <sub>f</sub>	PVX-P <sub>s</sub>	PVX-P <sub>f</sub>
Indirect ELISA on plates precoated with polyclonal antibodies	None	+	+	+	-	-	-
Indirect ELISA on non-precoated plates	None	-	+	-	-	+	+
Drop precipitin test	None	+	+	+	-	-	-
Dot-blot immunoassay	None	+	+	+	-	+	+
Dot-blot immunoassay	0.4% trypsin	+	+	-	-	+	+
Dot-blot immunoassay	5% pyrolicidine	-	-	+	-	+	+
Dot-blot immunoassay	2% SDS/5% 2-mercapto-ethanol, 3 min 100°C	-	-	(+)	-	+	+

\* The M and CsAg strains of PVX showed the same behaviour as the B strain with MAbs 58, 59, 66, 72 and 73. They failed to react, however, with MAbs 68, 69, 70 and 71.

† Tissue culture supernatants containing MAbs were used undiluted in the drop precipitin test and at a dilution of 1:10 in all other tests.

‡ Only MAb 70 showed this reactivity.

§ A strong reactivity was observed with MAbs 71 and 73, but only a weak one with MAb 72.

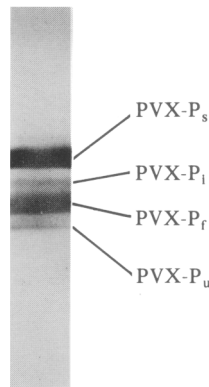


Fig. 2. Detection of PVX-P<sub>s</sub>, PVX-P<sub>i</sub>, PVX-P<sub>f</sub> and PVX-P<sub>u</sub> of the B strain of PVX with MAb 73 by electro-blot immunoassay. Electrophoretic migration was from top to bottom in a gel similar to that shown in Fig. 1.

Reactivity of determinants with MAb of group	Schematic representation of determinants (●) on undisturbed (a) and partially denatured (b) subunits	Properties of determinants
I		These determinants are located outside the protruding N terminus and are destroyed by extensive denaturation; they become masked in partially denatured preparations containing the protruding N terminus.
II		These determinants are located on the protruding N terminus; they are lost when the virus is treated with trypsin or proteases in crude plant sap; they become inaccessible or are destroyed in partially denatured preparations.
III		These determinants are located outside the protruding N terminus and are exposed in the B, CsAg and M strains only after partial denaturation of the virus particles. They are not destroyed by extensive denaturation, e.g. with SDS.

Fig. 3. Schematic interpretation of the results in Table 1.

CsAg and M strains only when the particles are, presumably, partially denatured by direct attachment to ELISA plates or nitrocellulose membranes. In the essentially undisturbed virus particles studied by indirect ELISA on plates precoated with polyclonal antibodies or in the drop precipitin test, these determinants are not recognized. They are possibly located close to the surface of the virus particles, and the finding by Torrance *et al.* (1986) that the group III MAbs did react with a number of other strains in indirect ELISA on plates precoated with polyclonal antibodies suggests that with these strains the determinants reacting with these MAbs may be freely accessible also in presumably undenatured virus particles. Alternatively, the crude sap preparations tested by Torrance *et al.* (1986) may have contained 'free' virus protein such as that described by Shalla & Shepard (1970). The determinants reacting with the MAbs of group III were not destroyed by extensive denaturation, e.g. by SDS treatment.

The conclusion that the MAbs of group III are specific for determinants outside the protruding N terminus is corroborated by the results of electro-blot immunoassay. The fact that these MAbs also reacted with PVX-P<sub>i</sub> and PVX-P<sub>v</sub> in addition suggests that the determinant(s) for which they are specific are not located in the C-terminal part of the protein.

The MAbs of group I are apparently specific for determinants which are exposed on the presumably undisturbed virus particles studied in indirect ELISA on plates precoated with polyclonal antibodies, or in drop precipitin tests. These determinants are not destroyed when the particles are attached directly to nitrocellulose membranes. When the particles are attached directly to ELISA plates these determinants become inactivated in preparations containing PVX-P<sub>s</sub>, but, surprisingly, not in preparations containing PVX-P<sub>f</sub>. Possibly these determinants become masked by the protruding N terminus in preparations containing PVX-P<sub>s</sub>. The loss of reactivity with group II MAbs indicates that the N terminus is denatured under these conditions. The determinants reacting with the MAbs of group I are destroyed by extensive denaturation, such as treatment with SDS or pyrrolidine, in contrast to those reacting with the MAbs of group III.

The MAbs in each of the three groups may not all recognize the same determinant. This seems to be true especially for MAb 71 which recognizes a determinant specific for the B strain, whereas MAb 72 and MAb 73 are reactive with determinants occurring in all three strains tested.

Differences in the reactivity of MAbs with virus preparations containing either intact or partially degraded protein subunits or 'free' virus protein may be important in field testing. Such variations in reactivity may possibly explain the differences in the detectability in purified and crude sap preparations of potato viruses Y and A which Boonekamp & Pomp (1985) observed in their studies with MAbs.

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#### REFERENCES

- AL MOUDALLAL, Z., BRIAND, J. P. & VAN REGENMORTEL, M. H. V. (1982). Monoclonal antibodies as probes of the antigenic structure of tobacco mosaic virus. *EMBO Journal* **1**, 1005-1010.
- AL MOUDALLAL, Z., ALTSCHUH, D., BRIAND, J. P. & VAN REGENMORTEL, M. H. V. (1984). Comparative sensitivity of different ELISA procedures for detecting monoclonal antibodies. *Journal of Immunological Methods* **68**, 35-48.
- BERCKS, R., KOENIG, R. & QUERFURTH, G. (1972). Plant virus serology. In *Principles and Techniques in Plant Virology*, pp. 460-499. Edited by C. O. Kado & H. O. Agrawal. New York: Van Nostrand Reinhold.
- BODE, L., BEUTIN, L. & KÖHLER, H. (1984). Nitrocellulose-enzyme linked immunosorbent assay (NC-ELISA) - a sensitive technique for the rapid visual detection of both viral antigens and antibodies. *Journal of Virological Methods* **8**, 111-121.
- BOONEKAMP, P. M. & POMP, H. (1985). The application of monoclonal antibodies in the diagnosis of agricultural and market garden crops. *Tijdschrift voor diergeneeskunde* **110**, 845.
- BURGERMEISTER, W. & KOENIG, R. (1984). Electro-blot immunoassay - a means for studying serological relationships among plant viruses? *Phytopathologische Zeitschrift* **111**, 244-250.
- CLARK, M. F. & ADAMS, A. N. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* **34**, 475-483.
- DOUGHERTY, W. G., WILLIS, L. & JOHNSTON, R. E. (1985). Topographic analysis of tobacco etch virus capsid protein epitopes. *Virology* **144**, 66-72.

- GALFRÈ, G. & MILSTEIN, C. (1981). Preparation of monoclonal antibodies: strategies and procedures. *Methods in Enzymology* **73B**, 3-46.
- GALFRÈ, G., MILSTEIN, C. & WRIGHT, B. (1979). Rat × rat hybrid myelomas and monoclonal anti-Fd portion of mouse IgG. *Nature, London* **277**, 131-133.
- JAEGLE, M. & VAN REGENMORTEL, M. H. V. (1985). Use of ELISA for measuring the extent of serological cross-reactivity between plant viruses. *Journal of Virological Methods* **11**, 189-198.
- KILMARTIN, J. V., WRIGHT, B. & MILSTEIN, C. (1982). Rat monoclonal anti-tubulin antibodies derived by using a new non-secreting rat cell line. *Journal of Cell Biology* **93**, 576-582.
- KOENIG, R. (1972). Anomalous behaviour of the coat proteins of potato virus X and cactus virus X during electrophoresis in dodecylsulfate containing polyacrylamide gels. *Virology* **50**, 263-266.
- KOENIG, R. (1978). ELISA in the study of homologous and heterologous reactions of plant viruses. *Journal of General Virology* **40**, 309-318.
- KOENIG, R. (1981). Indirect ELISA methods for the broad specificity detection of plant viruses. *Journal of General Virology* **55**, 53-62.
- KOENIG, R., TREMAINE, J. H. & SHEPARD, J. F. (1978). *In situ* degradation of the protein chain of potato virus X at the N- and C-termini. *Journal of General Virology* **38**, 329-337.
- LAEMMLI, U. K. & FAVRE, M. (1973). Maturation of the head of bacteriophage T4. I. DNA packaging events. *Journal of Molecular Biology* **80**, 575-599.
- MATTHEWS, R. E. F. (1948). Studies of potato virus X. II. Criteria of relationship between strains. *Annals of Applied Biology* **36**, 460-474.
- SHALLA, T. A. & SHEPARD, J. F. (1970). A virus induced soluble antigen associated with potato virus-X infection. *Virology* **42**, 1130-1132.
- SHEPARD, J. F. & SHALLA, T. A. (1972). Relative antigenic specificities of two PVX strains and their D-protein oligomers. *Virology* **47**, 54-60.
- TORRANCE, L., LARKINS, A. P. & BUTCHER, G. W. (1986). Characterization of monoclonal antibodies against potato virus X and comparison of serotypes with resistance groups. *Journal of General Virology* **67**, 57-67.
- TREMAINE, J. H., MacKENZIE, D. J. & RONALD, W. P. (1985). Monoclonal antibodies as structural probes of southern bean mosaic virus. *Virology* **144**, 80-87.

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