PARTIAL ANTIGENIC CHARACTERIZATION OF POTATO VIRUS S (ANDEAN STRAIN) BY MONOCLONAL ANTIBODIES

N. ČEŘOVSKÁ[1], M. FILIGAROVÁ[1], Z. ŠUBR[2]

[1]Institute of Experimental Botany, Academy of Sciences of Czech Republic, Na Karlovce 1, 160 00 Prague, Czech Republic;
[2]Institute of Virology, Slovak Academy of Sciences, Dúbravská cesta 9, 842 46 Bratislava, Slovak Republic

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Summary. – Four mouse monoclonal antibodies (MoAbs) against potato virus S Andean strain (PVS^A) were tested. While MoAbs 2 and 3 reacted only with complete virions and were apparently specific for epitopes dependent on quaternary structure, MoAbs 1 and 4 appared to be conformation independent and reacted with exposed regions on native virions as well as on the surface of dissociated coat protein subunits. This seems to be an evidence of metatope existence. The results of competitive binding tests together with reaction patterns of individual MoAbs suggest that the used MoAbs reacted with at least two different epitopes on PVS^A particles or polypeptide subunits. Immunoblot analysis of proteolytically cleaved PVS^A capsid protein (CP) confirmed close proximity of epitopes recognized by MoAbs 1 and 4. Anti-PVS polyclonal antibody recognized both intact CP and its natural or artificial digest, while the MoAbs bound to intact CP only. These results indicate that the surface virus-specific epitopes are located near the terminus of CP molecule as it is characteristic for potyviruses.

Key words: potato virus S; antigens; monoclonal antibodies

Introduction

PVS is a carlavirus affecting almost all cultivars of potatoes worldwide. Hinostroza-Orihuela (1973) and Santillan *et al.* (1980) described variant isolates of PVS from several South American potato cultivars that produced systemic mottle symptoms and necrosis in *Chenopodium quinoa* Wild., and were more efficiently transmitted by aphids or by contact than the previously studied isolates of PVS (PVS^O) commonly occuring European countries. The name Andean strain of PVS (PVS^A; Jones *et al.*, 1981) was introduced to distinguish isolates with such properties from PVS^O. Isolates of PVS^A have been found in some breeders• selections and potato cultivars in Germany and Netherlands (Dolby and Jones, 1987), and since they are more efficently transmitted by aphids and cause more severe symptoms on potato cultivars than PVS^O, the potential for spread to potato stocks in other European countries is a cause for concern. However, PVS^A is serologically indistinguishable from PVS^O in tests with polyclonal antisera (Weidemann and Koenig, 1990). For this reason MoAbs against PVS^A were produced (Čeřovská and Filigarová, 1995). In this study we attempted to extend the knowledge on the PVS^A antigenic properties using our four MoAbs in various types of ELISA and competitive binding tests, and in Western blot analysis of PVS^A CP and its proteolytic digests.

Abbreviations: ATA = antibody-trapped antigen; CBTs = competitive binding tests; CP = capsid protein; ELISA = enzyme-linked immunosorbent assay; MoAbs = monoclonal antibodies; PBS = phosphate buffered saline; PTA = plate-trapped antigen; PVS^A = potato virus S Andean strain; SDS-PAGE = SDS-polyacrylamide gel electrophoresis

Materials and Methods

Virus. PVS^A propagated in *Chenopodium quinoa* or *Lycopersicon esculentum* cv. Něvskij was purified as described by Čeřovská and Filigarová (1995). Purified virus was stored as a lyophylizate.

Viral-capsid protein was prepared by precipitating the viral nucleic acid by the mixture of guanidinium hydrochloride and lithium chloride (Wu and Bruening, 1971). Proteolytic cleavage by endoproteinase Gluc-C from *Staphylococcus aureus* strain V8 (V8-protease, Sigma), trypsin (Sigma), thermolysin (Serva) and chymotrypsin (Serva) was carried out as described by Šubr *et al.* (1993).

SDS-polyacrylamide electrophoresis (SDS-PAGE) of proteins was run according to Laemmli (1970).

MoAbs against PVS^ were prepared by immunization of Balb/c mice with native virus (Čeřovská and Filigarová, 1995).

Immunoblot analysis was carried out in a semidry system according to Hirano and Watanabe (1990). Nitrocellulose membranes (0.45 μm, Serva) were dried and stained immunospecifically as described by Šubr *et al.* (1993).

ELISA techniques (plate-trapped antigen – PTA ELISA and antibody-trapped antigen – ATA ELISA) were essentially those described by Čeřovská and Filigarová (1995).

Competitive binding tests (CBTs) were done with MoAbs biotinylated according to Bayer and Wilchek (1980). They were performed both in consecutive and simultaneous arrangement. Wells of microtiter plates were coated with anti-PVS IgG (1 μg/ml, 37°C, 4 hrs), washed, pure virus antigen (1 μg/ml) was added to each well and incubated overnight at 4°C. In consecutive CBT, first the unlabelled MoAb (2 hrs, 37°C) and then the biotinylated MoAb (2 hrs, 37°C) were added. In simultaneous CBT, instead of the last step the mixture of unlabelled and biotinylated MoAbs was added (4 hrs at 37°C). After an additional overnight incubation with the avidin-alkaline phosphatase conjugate and washing the substrate (4-nitrophenyl phosphate, Serva) was added. To assess a non-specific binding of biotinylated antibody, control wells were coated with PBS. In positive control, in which no competition occured, biotinylated antibodies were added to wells with PVS^ containing no competing antibodies. The absorbance of these wells was taken for 100% and used as a reference for estimation of the competition level of each unlabelled antibody.

Results and Discussion

Differentiation of PVS^ epitopes by ELISA

The epitope specificity of different MoAbs was determined by ELISA with the PVS^ virions or CP adsorbed to plates directly (PTA ELISA) or through polyclonal antibody (ATA ELISA). MoAbs 1 and 4 readily detected purified PVS^ in both PTA and ATA ELISA, whereas MoAbs 2 and 3 detected the virus only in ATA ELISA (Table 1). The influence of direct antigen binding onto the plastic plate surface on the disclosure of normally hidden epitopes is known (Joisson *et al.*, 1993). Thus while MoAbs 1 and 4 reacted with the epitopes present both on intact and altered virus particles, MoAbs 2 and 3 did so with epitopes occuring only on intact virus particles. The results obtained with the isolated CP subunits by the same ELISA techniques were in accordance with those mentioned above.

Table 1. Reactivity of MoAbs with virions (PVS^) and viral CP (PVS^-CP)in different ELISA techniques

MoAbs	ATA ELISA		PTA ELISA	
	PVS^	PVS^-CP	PVS^	PVS^-CP
1	+	+	+	+
2	+	–	–	–
3	+	–	–	–
4	+	+	+	+

A_{405} values higher than the threefold of background (+) or in the range of background (–).

Thus all epitopes were surface-located, those recognized by MoAbs 1 and 4 were contiguous metatopes, while those recognized by MoAbs 2 and 3 were discontiguous neotopes (Van Regenmortel, 1990; Šubr *et al.*, 1994).

Competition analysis

To obtain further information on epitope discrimination and topological epitope relationships, a series of CBTs was done. The results of simultaneous and consecutive CBTs were similar (Table 2). They revealed that the used MoAbs could be divided into two groups. MoAbs 1 and 4 competed with each other but they both did not compete with MoAbs 2 and 3 and vice versa.

An inhibition of antibody binding may occur due to a competition for the same epitope. Alternatively, an inhibition of binding of a second antibody specific for another epitope may occur if the epitopes are in close proximity or overlap. An inhibition may also be due to steric hindrance caused by the first antibody. Or, the first antibody may prevent the access for a second antibody by altering the con-

Table 2. Competitive binding tests with MoAbs to PVS^

Unlabelled MoAbs	Biotinylated MoAbs			
	1	2	3	4
1	0/20	79/85	100/100	0/0
2	90/90	0/0	35/38	80/85
3	80/75	0/0	0/0	70/78
4	0/0	65/70	100/90	0/15

A_{405} was recorded after incubation at 37°C after 60 mins. The values represent ratios of activities (%) remaining after blocking in consecutive/simultaneous CBT. 100% = activity of the sample without blocking.

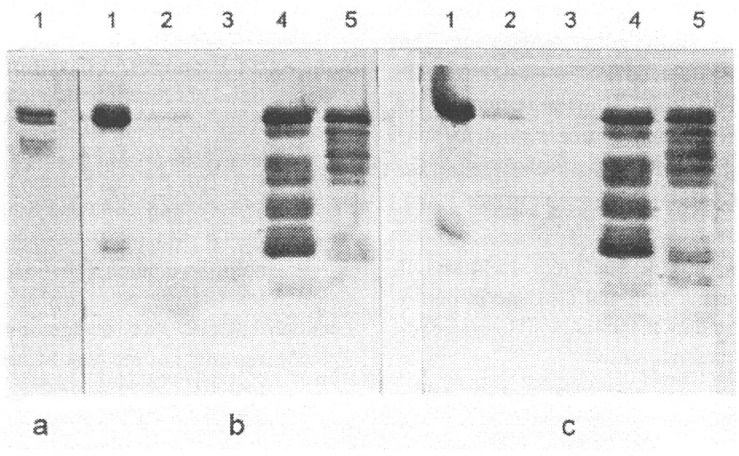

Fig. 1
Immunoblot analysis of PVS^A CP
Rabbit polyclonal antibody against PVS (Čeřovská and Filigarová, 1995) (a), anti-PVS^A MoAb 1 (b) and anti-PVS^A MoAb 4 (c). CP uncleaved (lanes 1) or cleaved by V8-protease (lanes 2), trypsin (lanes 3), chymotrypsin (lanes 4) and thermolysin (lanes 5).

formation of the second epitope or even the entire antigen (Kubanek *et al.*, 1991).

No mutual influence between both pairs of MoAbs indicates that the binding sites of these MoAbs are not topologically related and no conformational changes of MoAbs 1 and 4 binding sites by binding of MoAbs 2 and 3 occur. Thus the two groups of epitopes are spacially distant on the mature CP. On the contrary, MoAbs 1 and 4 recognized topologically very closely related or identical epitopes as did MoAbs 2 and 3 too. However, discrete epitopes may occur close each to other and behave as one site in CBT (Schmaljohn *et al.*, 1983).

Table 2 also shows that MoAb 3 completely blocked the binding of MoAb 2 while MoAb 2 blocked the binding of MoAb 3 only by about 60%. Such a result may reflect greater affinity of MoAb 3 or different actual concentration of specific IgG in respective MoAb preparations (MoAbs were not affinity-purified).

Competing antibodies can be further characterized by comparison of competition curves obtained in assays performed by simultaneous and consecutive procedures. Competition curves are influenced by the affinity and concentration of the unlabelled antibody relative to the labelled antibody. A comparison of simultaneous and consecutive competition curves suggests which of these factors affect more significantly the competition ability of the unlabelled antibody (Brezovsky and Berkower, 1984; Hosang, 1985).

In our case, both pairs of MoAbs gave gradually growing competition curves in both simultaneous and consecutive CBTs (data not shown). Thus the mutual competition of respective MoAbs was rather more affinity – then concentration-based.

Immunoblot analysis

The results of immunoblot analysis fitted those obtained by ELISA. While MoAbs 1 and 4 reacted with the denaturated PVS^A CP immobilized on nitrocellulose membrane (Fig. 1b,c), MoAbs 2 and 3 did not.

The proteolytic cleavage of CP followed by immunoblot analysis with MoAbs 1 and 4 showed no difference between peptide patterns obtained. This fact proved that these MoAbs recognized either very close (probably overlapping) epitopes or even the same epitope. V8-protease or trypsin cleavage of CP destroyed these epitopes (Fig. 1b,c).

In SDS-PAGE the PVS^A CP migrated as two double bands of 39K and 35K, respectively. The faster protein was apparently produced by proteolysis of CP *in vitro* and/or *in vivo*. This was proved by excision of respective bands from the gel and their subsequent proteolysis by V8-protease resulting in identical peptide patterns (data not shown).

The rabbit polyclonal antibody against PVS reacted in immunoblot analysis both with slow and fast zones (Fig. 1a). However, MoAbs 1 and 4 bound only to the slower zone corresponding to intact CP.

A similar phenomenon is known in potyviruses, where significant proteolysis occurs *in vitro*, leading to a loss of both N- and C-termini of CP, normally exposed on the surface of native virions. In such a way, potyviruses lose their virus-specific epitopes. However, the resulting modified viral particles preserve their reactivity with broadly (group) specific antisera (Shukla *et al.*, 1988; Shukla and Ward, 1989).

Our results may indicate an analogical CP processing for the viruses of the *Carlavirus* genus, particularly PVS^A. One or both CP terminal regions probably bear most of immu-

noepitopes, because the reactivity of the polyclonal antibody with processed CP was significantly weaker than that with intact CP. On the other hand, PVS^{A}-specific MoAbs bound to intact CP only. Thus the CP arrangement in the capsid of carlaviruses seems to share some similarity with that of potyviruses although no serological relationship between these viral taxa does exist.

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References

Bayer EA, Wilchek M (1980): The use of avidin-biotin complex as a tool in molecular biology. *Methods Biochem. Anal.* **26**, 1–45.

Brezovsky JA, Berkower IJ (1984): Antigen-antibody interactions. In Paul WE (Ed.): *Fundamental Immunology*. Raven Press, New York, pp. 595–644.

Čeřovská N, Filigarová M (1995): Specific detection of the Andean strain of potato virus S by monoclonal antibodies. *Ann. Appl. Biol.* **127**, 87–93.

Dolby CA, Jones RAC (1987): Occurrence of the Andean strain of potato virus S in imported potato material and its effects on potato cultivars. *Plant Pathol.* **36**, 381–388.

Hinostroza-Orihuela AM (1973): Some properties of potato virus S isolated from peruvian potato varieties. *Potato Res.* **16**, 244–250.

Hirano H, Watanabe T (1990): Microsequencing of proteins electrotransferred onto immobilizing matrices from polyacrylamide gel electrophoresis: application to an insoluble protein. *Electrophoresis* **11**, 573–580.

Hosang M (1985): Surinam binds to platelet-derived growth factor and inhibits its biological activity. *J. Cell. Biochem.* **29**, 265–273.

Joisson C, Kuster F, Plaue S, Van Regenmortel MHV (1993): Antigenic analysis of bean pod mottle virus using linear and cyclized synthetic peptides. *Arch. Virol.* **128**, 299–317.

Jones RAC, Fribourg CE, Slack SA (1981): Potato virus and virus-like diseases. In Barnet OW and Tollin SA (Eds): *Plant Virus Slide Series Set No.2.* Clemson University, Clemson, SC., p. 59.

Kalmakoff J, Parkinson AJ, Crawford AM, Williams BRG (1977): Solid phase radioimmunoassays using labelled antibodies: a conceptual framework for designing assays. *J. Immunol. Methods* **14**, 73–84.

Kubanek CA, Hill JH, Van Deusen RA, Durand DP (1991): Consecutive and simultaneous competition ELISA of monoclonal antibodies: Characterization of epitope relationships or immunoglobulin idiotypes. *J. Phytopathol.* **133**, 99–104.

Laemmli UK (1970): Cleavage of structural proteins during the assembly of the heat of bacteriophage T4. *Nature* **227**, 680–685.

Santillan FW, Fribourg CE, Jones RAC (1980): Estudio comparativo de once aislamiento de virus S der la papa de la region Andiana. *Fitopatologia* **15**, 42–43 (in Spanish).

Schmaljohn AL, Kokubun KM, Cole GA (1983): Protective monoclonal antibodies define maturational and pH-dependent antigenic changes in Sindbis virus E1 glykoprotein. *Virology* **130**, 144–154.

Shukla DD, Strike PM, Tracy SL, Gough KH, Ward CW (1988): The N and C termini of the coat proteins of potyviruses are surface-located and the N terminus contains the major virus-specific epitopes. *J. Gen. Virol.* **69**, 1497–1508.

Shukla DD, Ward CW (1989): Identification and classification of potyviruses on the basis of coat protein sequence data and serology. *Arch. Virol.* **106**, 171–200.

Šubr Z, Gallo J, Matisová J (1993): Some properties of coat proteins of two comoviruses. *Acta Virol.* **37**, 47–53.

Šubr Z, Gallo J, Matisová J (1994): Characterization of monoclonal antibodies against broad bean stain and red clover mottle viruses. *Acta Virol.* **38**, 317–320.

Van Regenmortel MHV (1990): The structure of viral epitopes. In *Immunochemistry of Viruses. II. The Basis for Serodiagnosis and Vaccines.* Elsevier, Amsterdam, pp. 1–24.

Weidemann HL, Koenig R (1990): Differentiation of isolates of potato virus S which infect Chenopodium quinoa systematically by means of quantitative cDNA hybridization tests. *J. Plant Dis. Prot.* **97**, 323–327.

Wu G-J, Bruening G (1971): Two proteins from cowpea mosaic virus. *Virology* **46**, 596–612.