MONOCLONAL ANTIBODIES AGAINST POTATO VIRUS A – IMMUNOBLOT ANALYSIS

Z. ŠUBR[1], T. MORAVEC[2], N. ČEŘOVSKÁ[2]

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

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Summary. – Monoclonal antibodies (MoAbs) against potato virus A (PVA) were examined in their reactivity with PVA and its denatured capsid protein (PVA-CP) bound to the nitrocellulose membrane. Five MoAbs reacted with native PVA, three of them also with PVA-CP. One MoAb gave no reaction in dot-blot test. In polyacrylamide electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE) PVA-CP migrated as two major bands. In immunoblot analysis, two MoAbs reacted only with the slower band, one only with the faster one. We presume that those bands do not correspond to the intact CP but they do to truncated N- and C-terminal CP molecules, respectively, and that the corresponding epitopes reacting with MoAbs are localized near to both termini of CP molecules. After mild trypsinolysis of PVA particles no MoAb reacted with resulting "core" CP.

Key words: potato virus A; monoclonal antibodies; epitopes; immunoblot analysis; proteolysis

Introduction

PVA (family *Potyviridae*, genus *Potyvirus*) belongs to economically important potato patogens. For effective field diagnosis in Czech Republic a panel of MoAbs has been recently prepared. The latter have been characterized in various ELISA and competition binding tests, and their cross-reactivity with some other potyviruses has been examined (Čeřovská and Filigarová, 1992). In this paper, we focused on the reactivity of these MoAbs with PVA CP in immunoblot analysis.

Materials and Methods

Virus. The isolate PVA-LI was grown and purified as described by Čeřovská and Filigarová (1992).

MoAbs (IgG) were described earlier (Čeřovská and Filigarová, 1992).

SDS-PAGE was done according to Laemmli (1970) in 14% gels. A mixture of size marker proteins (Pharmacia) was used for estimation of relative molecular mass (M_r). Gels were silver-stained (Marcinka *et al.*, 1992).

Dot-blot tests and immunoblot analysis. In dot-blot tests, 1 µl of antigen (native or SDS/heating-denatured PVA) was dropped onto a piece of nitrocellulose membrane (Serva, 0.45 µm) and dried for 2 hrs at 37°C. Electroblotting of proteins was done in a semidry system (Hirano and Watanabe, 1990). After drying, the membranes both for dot-blot and immunoblot analysis were further processed as described before (Šubr *et al.*, 1993).

Proteolysis of native viral particles with trypsin (Sigma) was performed according to Shukla *et al.* (1988).

Peptide mapping. PVA CP was cleaved with trypsin, chymotrypsin, V8-protease and thermolysin (Serva) as described by Šubr *et al.* (1993).

Results and Discussion

We examined first the MoAbs for their reactivity with native and denaturated PVA-CP in dot-blot tests (Table 1). Only three MoAbs (151, 290, 328) reacted with the

Abbreviations: CP = capsid protein; MoAb = monoclonal antibody; PVA = potato virus A; PVA-CP = PVA capsid protein; SDS = sodium dodecyl sulphate; SDS-PAGE = polyacrylamide electrophoresis in the presence SDS

Table 1. Reactivity of MoAbs with native PVA and denatured PVA-CP in dot-blot test

MoAb	Native PVA	Denatured PVA-CP
151	+	+
187	+	–
290	+	+
328	+	+
534	–	–
634	+	–

denatured PVA-CP, which correlated with the ELISA results obtained before (Čeřovská and Filigarová, 1992). Surprising was the fact that MoAb 534 did not react in dot-blot test. The antigen binding to the nitrocellulose membrane can probably affect the exposed epitopes in some way, similar to the binding of the virus to the surface of plastic wells in ELISA (Wang *et al.*, 1992).

Only MoAbs 151, 290 and 328 were used to detect PVA-CP in immunoblot analysis. In SDS-PAGE the CP of freshly prepared PVA migrated as two major bands of M_r of 33.1 K and 28.8 K, respectively. After blotting onto nitrocellulose membrane, only the upper band reacted with MoAbs 151 and 328, and only the lower one with MoAb 290 (Fig. 1). This result made doubtful our first hypothesis that the faster form of CP corresponded simply to the truncated molecule of CP as it was recently observed in the case of potato virus S, a carlavirus (Čeřovská *et al.*, 1996).

According to another interpretation the two bands were two redox forms with slightly different conformation and epitope exposition even in conditions of SDS-PAGE. E.g., Boonekamp *et al.* (1990) observed a PVA epitope with MoAb binding ability strongly dependent on some plant oxidating substance. In our case, however, when different concentrations of 2-mercaptoethanol (in the range of 0 – 10%) were introduced into the sample, the resulting electrophoretic patterns remained unchanged (data not shown). This result indicated that this interpretation should be rejected.

We propose that the both observed bands correspond to proteolytically processed forms of the CP molecule, one of them shortened at the N-terminus, the other one at the C-terminus. The respective epitopes must be localized on the opposite ends of PVA-CP. They are both exposed on the surface of mature viral particles (Shukla *et al.*, 1988). Competition binding test data confirm mutual spatial proximity of epitopes corresponding to MoAbs 151, 290 and 328 (Čeřovská and Filigarová, 1992).

Both the N- and C-termini of different potyviruses are known to bear virus-specific surface epitopes. They may be removed by mild proteolysis of virions, while group-specific epitopes, conserved in many potyviruses, remain preserved

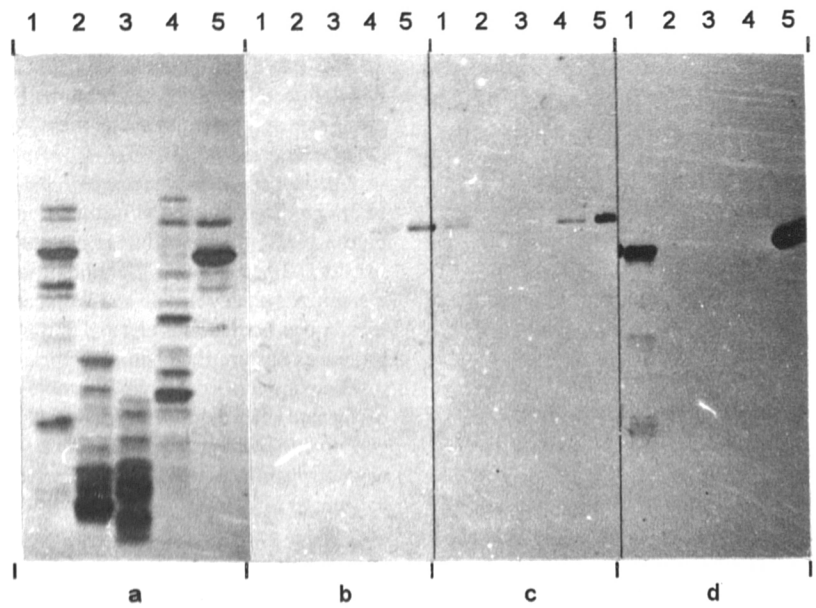

Fig. 1
SDS-PAGE peptide mapping of freshly prepared PVA-CP
Silver-stained gel (a) and immunoblots with MoAbs 151 (b), 328 (c), and 290 (d), respectively. PVA-CP cleaved with thermolysin (lanes 1), chymotrypsin (lanes 2), trypsin (lanes 3) and V8-protease (lanes 4), and PVA-CP not treated with a protease (lanes 5).

on the particles with such modified "core" CP (Shukla *et al.*, 1988; Shukla and Ward, 1989).

According to our interpretation the full-length CP was not detected in our SDS-PAGE experiments. It might be fully disintegrated in the course of the purification process. The Pepscan test is probably the only means to decide whether our hypothesis is true or not. So far, its application to the PVA-CP did not to find any epitope on CP close to its C-terminus using monoclonal as well as polyclonal antibodies. However, but the N-termini of intact as well as "core" CP molecules have been shown immunodominant (Andreeva *et al.*, 1994).

When the purified PVA was stored at 4°C for 2 – 5 months, four minor bands became visible in the SDS-PAGE pattern of PVA-CP, but none of them reacted with either MoAb (Fig. 2). Their M_r were 29.2 K, 28.1 K, 27.2 K and 26.3 K, respectively.

The trypsinolysis of PVA particles resulted in a complete conversion of CP into its fastest form (26.3 K) (Fig. 2). Probably both original one-end-truncated molecules converted into identical both-ends-truncated "core" molecules. No MoAb reacted with the "core" CP either in immunoblot analysis (Fig. 2) or in plate-trapped antigen ELISA. Our idea about spontaneous PVA-CP proteolysis is illustrated in Fig. 3.

On the basis of M_r of the individual fragments we presume the intact PVA-CP of about 35.6 K. However, we never observed such a band in SDS-PAGE even with freshly purified PVA. The both termini of CP molecule look like very easy to break off either in the course of the sample preparation (heating, denaturation) or purification. Our MoAbs did not bind to peptides obtained by different protease treatment of PVA-CP with exception of weak reaction of MoAb 290 with some thermolysin-digests of CP (Fig. 1). This fact may also indicate high susceptibility of PVA-CP termini to proteolytic treatment in general.

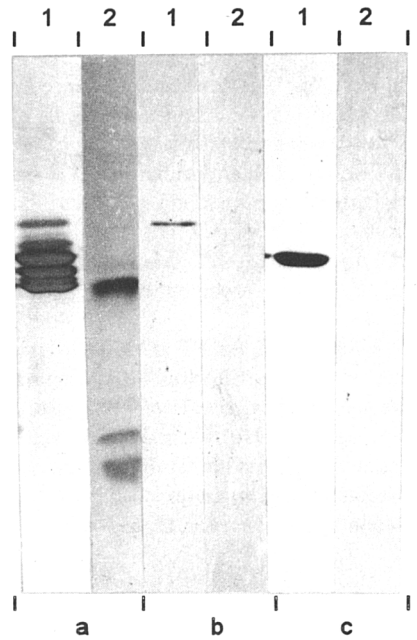

Fig. 2
Effect of mild trypsinolysis of PVA virions on the SDS-PAGE mobility of viral CP
Silver-stained gel (a) and immunoblots with MoAbs 151 (b) and 290 (c), respectively. PVA (the same preparatin as in Fig. 1, stored for 3 months at 4°C) (lanes 1) and PVA treated with trypsin as described by Shukla *et al.* (1988) (lanes 2).

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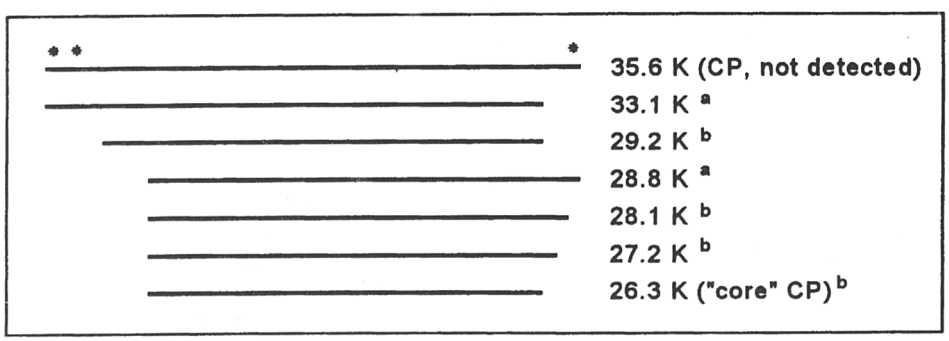

Fig. 3
Scheme of proposed spontaneous proteolytic processing of PVA-CP
Rough localization of epitopes recognized by MoAbs 151 and 328 (**) and MoAb 290 (*). The hypothesis does not include information on the N-C-orientation of the molecule. [a]Forms present in freshly purified PVA preparations. [b]Forms occuring in the course of PVA storage at 4°C.

References

Andreeva L, Järvekülg L, Rabenstein F, Torrance L, Harrison BD, Saarma M (1994): Antigenic analysis of potato virus A particles and coat protein. *Ann. Appl. Biol.* **125** 337–348.

Boonekamp PM, Pomp H, Gussenhoven GC (1990): Production and characterization of monoclonal antibodies to potato virus A. *J. Phytopathol.* **128**, 112–124.

Čeřovská N, Filigarová M (1992): Monoclonal antibodies against potato virus A – competitive binding tests. *Acta Virol.* **36**, 497–504.

Čeřovská N, Filigarová M, Šubr Z (1996): Partial antigenic characterization of potato virus S (Andean strain) by monoclonal antibodies. *Acta Virol.* **40**, 23–26.

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.

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

Marcinka K, Röhring, C, Kluge, S (1992): Changes in protein patterns of pea plants systemically infected with red clover mottle virus. *Biochem. Physiol. Pflanzen* **188**, 187–193.

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.

Wang G, Porta C, Chen Z, Baker TS, Johnson JE (1992): Identification of FAb interaction footprint site on an icosahedral virus by cryoelectron microscopy and X-ray crystallography. *Nature* **355**, 275–278.