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Analysis of antigenic structure of Potato Virus M Ukrainian strains

S. S. Viter, T. Yu. Tkachenko, L. P. Kolomietz[1], Yu. L. Radavsky

Institute of Bioorganic and Petroleum Chemistry of Ukrainian National Academy of Sciences
1 Murmanska str., Kyiv, 02094, Ukraine

[1]Institute of Agricultural Microbiology of Ukrainian National Agricultural Academy of Sciences
97 Shevchenko str., Chernigov, 14027, Ukraine

The antigenic structure of two Ukrainian strains U1 and U7 of Potato Virus M (PVM) was studied by using three mouse monoclonal antibodies (MAbs). Tryptic fragments of PVM coat protein (CP) ^{22}Glu-Lys^{35} and ^{22}Gly-Lys^{35} were found to be recognised by two MAbs, M6D5 and M9G1. The amino acid substitution Glu → Gly at the position 22 of PVM U7 coat protein did not affect antibody binding to both tryptic fragments and PVM CP. Mab M9G1 interfered with MAb M4C1 in dot immunobinding assay. Synthetic peptide P14, corresponding to tryptic fragment ^{22}Glu-Lys^{35}, inhibited the interaction of MAb M6D5 with CP and peptide P14 more effectively than that of MAb M9G1. The modification of the side chain positive charge of Lys^{35} to negative one in tryptic peptides ^{22}Glu-Lys^{35} and ^{22}Gly-Lys^{35} using citraconic anhydride resulted in a two-fold increase of the reaction of MAb M9G1 and slightly reduce the interaction of MAb M6D5 with both fragments. On the basis of these results, it was concluded that three PVM-specific epitopes are located at the N-terminal region and form original immunogenic site of PVM coat protein. MAbs M6D5 and M9G1 recognise sequentially overlapping epitopes and the common site for both epitopes is presented in the fragment ^{22}Glu/Gly-Lys^{35}. MAbs M4C1 and M9G1 recognise either overlapping, or conformational approximated epitopes.

Introduction. Potato Virus M (PVM) is a member of the carlavirus genus. Virus particles have filamentous shape with 650 nm in length and 13 nm in diameter and compose of multiple copies of the coat protein (CP) with molecular mass of approximately 36 kDa and a monopartite plus-sense single stranded RNA [1, 2]. The complete nucleotide sequence of PVM Russian strain [3] and partial 3'-end sequence of German strain genomic RNA [4] have been established earlier. PVM genomic RNA consists of six different size open reading frames, four non-coding regions and 3'-terminal poly(A) tract [3]. It was shown that the 3'-terminal regions of genomic RNA of all carlaviruses and potexviruses have similarity with PVM in their gene arrangement [5].

PVM infects many hosts but infection in *Solanum tuberosum* is of major economic importance. PVM may cause a crop loss up to 70 % and tubers quality decrease, although the infection is symptomless or displayed as mild leaf rolling in the hosts. PVM is mechanically transmissible and has aphid vectors, being transmitted in the non-persistent manner [6].

Over the last years the genome organization and encoded proteins of the carlaviruses have been studied and presented in the review [5]. However, little is known about antigenic structure of the carlaviruses, and particularly of PVM. The antigenic structure of PVM Russian strain has been carried out and location of PVM-specific epitope and its primary structure were established [7]. In the study of Foster and Mills [8], polyclonal antibodies have been used to compare antigenic properties of several carlaviruses and two strains of Potato Virus S (PVS), Andean and Ordinary, which are different in their biological properties. Various degrees of antigenic relationships among some members of the carlaviruses were found by serological tests, but no substantial differences between PVS strains were established. In another study [9], monoclonal antibodies (MAbs) against PVS Andean strain have been used to determine the location of antigenic sites in the CP subunit. It was

shown that the PVS-specific epitopes are surface-exposed and located at the N- and C-termini of protein molecule, similarly to the antigenic sites of potyviruses' CP. Adams and Barbara [10] studied recognition of seven carlaviruses by antisera generated against 16 carlaviruses and seven other filamentous viruses. The heterogeneity of antigenic relationships among the seven carlaviruses has been shown in that study, but no specific reaction of carlaviruses with antisera against non-carlaviruses was found. Monoclonal antibodies against the PVM Russian isolate were applied to analyse the cross-reactivity among 15 isolates from Finnish collection as well as Russian, Hungarian and German isolates [11]. In this study, all antibodies reacted with 18 PVM isolates identically, suggesting the presence of common and immunologically conserved epitopes in the PVM isolates.

In the present study, we analysed the antigenic structure of two PVM Ukrainian strains, U1 and U7, using three MAbs. We also used tryptic and synthetic peptides to characterise overlapping epitopes in two ELISA formats.

Materials and Methods. *Virus purification.* PVM U1 and U7 strains were propagated on *Lycopersicon esculentum* cv. Pobeda. The leaves of infected plants were harvested in 4 weeks after infection and viruses were purified as described in [12], using 50 mM KH_2PO_4-KOH buffer with 5 mM EDTA and 0.2 % 2-mercaptoethanol, pH 7.5, and 0.3 M glycine-KOH buffer, pH 8.5. All purification procedures were performed at 4 °C. Virus concentration was estimated by absorbance at 260 nm, using relationship 2.88 A_{260} = 1 mg/ml. Ratios $A_{260/280} = 1.21$, $A_{max/min} = 1.12$ [2] and SDS-electrophoresis [13] were used to analyse purity and quality of virus preparations. Purified viruses were stored at −20 °C in 0.3 M glycine-KOH buffer, pH 8.5, containing 50 % glycerol.

Coat protein preparation. PVM CPs U1 and U7 were prepared as described in [14]. Purified viruses at concentration 3—5 mg/ml in the 0.3 M glycine-KOH buffer, pH 8.5, were treated with mixture of guanidine-HCl and LiCl at final concentrations 4 M and 2 M, respectively, followed by freezing at −70 °C for 3 h and thawing at room temperature. Precipitated virus RNA was collected by centrifugation at 5000 *g* for 30 min. Supernatant, containing CP, was dialysed against ammoniac water, pH 8, and lyophilised. Protein concentration was estimated according to [15].

SDS-PAG-electrophoresis. PVM CPs were analysed by SDS-PAGE on a 10.5 % gel with 0.1 % SDS according to [13]. Protein samples were prepared in 10 mM Tris-HCl buffer, pH 8, containing 1 mM EDTA, 1 % SDS and 5 % 2-mercaptoethanol. Electrophoresis was performed in Tris-glycine running buffer, pH 8.3, containing 0.1 % SDS. LMW SigmaMarkers («Sigma», USA) were used to determine the molecular mass of coat protein: α-lactalbumin 14,2 kDa, trypsin inhibitor 20 kDa, trypsinogen 24 kDa, carbonic anhydrase 29 kDa, glyceraldehyde-3-phosphate dehydrogenase 36 kDa, ovalbumin 45 kDa, BSA 66 kDa. Gels were stained using a Silver Stain Plus kit according to the manufacturer protocol.

Proteolytic cleavage of PVM coat protein. CPs U1 and U7 were separately digested with trypsin («Sigma») in 0.1 M ammonium bicarbonate, pH 8, at 37 °C for 4 h at an enzyme-substrate ratio 1 : 50 (w/w). Reaction was stopped by adding 0.1 % acetic acid lowering pH to 2.5. Trypsin-treated proteins were stored at −20 °C.

Fractionation of tryptic peptides and sequencing. Tryptic mixtures of CPs U1 and U7 were fractionated by high-performance liquid chromatography (HPLC) on a C18 LiChrosorb RP-18 column (5 μm, 4.6 mm × × 250 mm, LKB, Sweden) using the 2—60 % linear gradient of acetonitrile («Fluka», Switzerland) in 0.1 % trifluoroacetic acid (TFA) at a flow rate of 1.0 ml/min. Optical density was monitored at 206 nm and 280 nm. Collected fractions were dried and peptide material of each fraction was dissolved in appropriate buffer before using in procedures.

Tryptic fractions found to be recognised by MAbs were repeatedly fractionated using the same column and combination of linear (2—40 %) and isocratic acetonirtile gradients in 0.1 % TFA. Amino acid sequence analysis was performed in a gas sequenator, model 816 («Knauer», Germany), according to the manufacturer protocol.

Peptide synthesis. Synthetic peptides P14 (H_2N-^{22}EARPLPTAADFEGK^{35}-COOH) and H_2N-^{154}KDASSSVF^{161}-COOH as negative control were synthesized (NPO Verta, St.-Petersburg, Russia) by solid phase method using Boc-protected amino acids, according to [16].

ELISAs. Lyophilised protein and vacuum dried peptide samples were dissolved in phosphate buffered saline: 10 mM Na_2HPO_4-NaH_2PO_4 buffer, pH 7.3, containing 0.14 M NaCl (PBS). Microtiter plates («Nunk», Denmark) were coated with antigens by evaporating of PBS at 37 °C overnight. Antigen-coated wells were rinsed three times with PBS and blocked with 1 % BSA in PBS, containing 0.05 % Tween-20 (PBST), for 4 h at 37 °C. MAbs were diluted in PBST with 0.3 % BSA and incubated with antigens for 2 h at 37 °C. Then plates were washed five times with PBST and rabbit anti-mouse IgG-horse radish peroxidase (HRP) conjugate (1 : 2000) in PBST with

0.3 % BSA was added. Plates were incubated at 37 °C for 1.5 h and washed five times with PBST. Colour reaction was developed by adding substrate buffer (0.1 M citrate-0.15 M Na_2HPO_4, pH 4.5), containing 0.6 mg/ml o-phenylenediamine («Fluka», Switzerland) and 0.01 % H_2O_2. Absorbance was measured at 450 nm using Kinetic Microplate Reader (Molecular Devices, «Fisher», USA).

In inhibition ELISA experiments, MAbs were incubated with two-fold decreasing concentrations of synthetic peptide P14 for 1 h at room temperature. Then these mixtures were added to the precoated antigens CP U1 — 500 ng (14 pM) per well and peptide P14 — 50 ng (33 pM) per well with following incubation for 1 h at 37 °C. The other steps of inhibition ELISA were performed as describe above in ELISA procedure.

Citraconilation of peptides. Tryptic peptides ^{22}Glu-Lys^{35} (U1—14) and ^{22}Gly-Lys^{35} (U7—16) at concentration 4 nM each were treated with 50-fold molar excess of citraconic anhydride («Fluka») in 0.2 M $NaHCO_3$, pH 9.5, for 2 h at room temperature. Intact and modified peptides at concentration 0.5 nM per well each were analysed in ELISA.

Dot immunobinding assay. Protein samples were dotted on nitrocellulose membrane strips (Hybond C, 0.45 μm, «Amersham», Great Britain). Strips were equilibrated 3 × 10 min with PBS, pH 7.3, and blocked with 3 % BSA in PBST at 37 °C overnight. Then strips were first incubated with MAbs M6D5 (1 : : 1000) or M9G1 (1 : 500) in PBST with 1 % BSA for 2 h at 37 °C, washed 5 × 10 min with PBST and then incubated with MAb M4C1-HRP conjugate (1 : 500) for 2 h at 37 °C. After incubation with conjugate, strips were washed 5 × 10 min with PBST. Colour reaction was developed with 0.025 % 3,3'-diaminobenzidine («Sigma») and 0.01 % H_2O_2 in PBS and stopped by rinsing nitrocellulose strips in distilled water.

Monoclonal antibodies. MAbs M6D5, M4C1 and M9G1 against PVM Russian strain were raised against native virus particles of PVM Russian strain and have been characterised earlier [11]. Professor M. Saarma, Institute of Chemical Physics and Biophysics (Estonia), kindly provided antibodies and MAb M4C1-HRP conjugate for this study.

Results and Discussion. Two serologically related PVM strains U1 and U7 were isolated in Ukraine and differentiated in monolines by multiple passages through the indicator plant *Chenopodium murales*. These strains have different biological properties, particularly, pathogenic features and symptoms they cause in the host and indicator plants. Potato plants infected with mild strain PVM U1 no symptoms were found, while the mild necrotic spots (diameter 1—3 mm) with diffusive borders were usually observed on the indicator plant *C. murales*. Severe strain PVM U7 causes strong leaf rolling in the potato plants and provokes the high expression of necrotic spots (diameter 2—4 mm) with sharp borders on the indicator plant *C. murales*. PVM mild strain U1 has been successfully used as vaccine strain for cross-protection of potato plants against severe strain PVM U7.

The SDS-electrophoresis results showed small differences in apparent molecular mass for CPs U1 — 37.3 kDa and U7 — 36.4 kDa (Fig. 1). Various substitutions, deletions and post-translational modifications in protein sequence may explain this difference between molecular mass of studied CPs. It is well known that post-translational modifications of the polypeptide backbone, such as O- and N-glycosylation, may cause an anomalous migration of the intact proteins in the SDS-PAGE [17]. A degree and manner of glycosylation may also influence protein

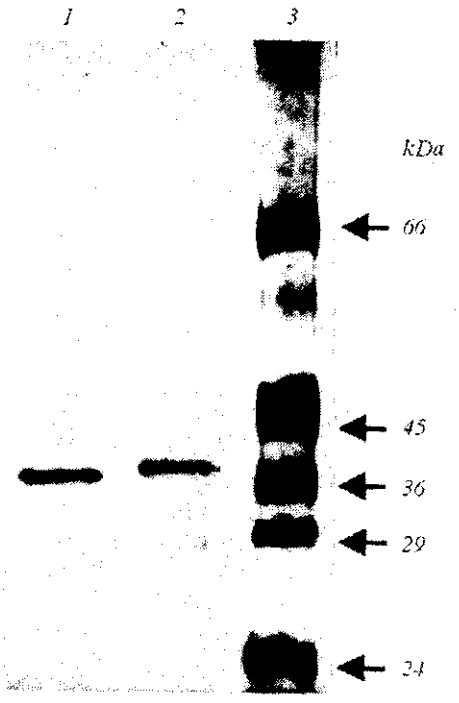

Fig. 1. Electrophoresis of PVM U1 and U7 coat proteins CP) in 10.5 % polyacrylamide gel with 0.1 % SDS according to Laemmli [13]. Lines: *1* — PVM U7 CP; *2* — PVM U1 CP; *3* — molecular mass markers (LMW SigmaMarkers): α-lactalbumin — 14.2 kDa; trypsin inhibitor — 20 kDa; trypsinogen — 24 kDa; carbonic anhydrase — 29 kDa; glyceraldehyde-3-phosphate — 36 kDa; egg albumin — 45 kDa; bovine serum albumin — 66 kDa

apparent molecular mass. Such variations in protein modification may result in different values of molecular mass, deduced from the CP gene of PVM, 34 kDa [3], and observed for CPs U1 and U7 in SDS-electrophoresis. Recently, O-glycosylation has been shown for all known strains of Potato Virus X [18]. However, glycosylation of carlaviruses' CPs have not yet been studied.

To localise specific epitopes, the CPs U1 and U7 were digested with trypsin. The cleavage products were fractionated by HPLC in the linear acetonitrile gradient. Collected fractions were analysed by ELISA using three MAbs M4C1, M6D5, and M9G1. The results showed that: 1) no fraction was recognised by MAb M4C1; 2) MAb M6D5 reacted with fractions 13 and 14 of CP U1 (U1—13 and U1—14) and fractions 15 and 16 of CP U7 (U7—15 and U7—16); 3) MAb M9G1 recognised only U1—14 and U7—16.

Antibody-recognised fractions were additionally purified by HPLC using a combination of the linear and isocratic acetonitrile gradients. Purified peptides were again analysed in ELISA and their primary structures were established on a gas sequenator:

U1-13: ^{22}EARPLPTAADFEGKDTSENTDGR^{44};
U1-14: ^{22}EARPLPTAADFEGK^{35};
U7-15: ^{22}GARPLPTAADFEGKDTSENTDGR^{44};
U7-16: ^{22}GARPLPTAADFEGK^{35}.

The sequences of peptides U1—14 and U7—16 correspond to the sequence at the positions 22—35 (further in the text as ^{22}Glu/Gly-Lys^{35}) and peptides U1—13 and U7—15 — to the sequence at the positions 22—44 (further in the text as ^{22}Glu/Gly-Arg^{44}) of the PVM Russian strain CP [3]. Peptides U1—13 and U7—15 are lengthened by nine residues at the C-terminus in comparison with peptides U1—14 and U7—16 and contain the non-cleaved bond ^{35}Lys-Asp^{36}. The incomplete cleavage of peptide bond Lys-Asp may be due to a slowing down of trypsin hydrolysis in the presence of acidic residue or the enzyme access to this bond is limited by steric hindrances on the protein surface. The CP U7 was found to have the amino acid substitution Glu → Gly at the position 22 that did not affect the reaction of MAbs with peptides. MAb M6D5 recognized four peptides, while MAb M9G1 reacted only with the two short peptides. Other investigators [19, 20] have observed a similar phenomenon, when antibody recognised a shorter peptide, but not longer one. On the basis of these ELISA results we assumed that, first, MAbs M6D5 and M9G1 recognise two overlapping epitopes (M6D5 epitope and M9G1 epitope, respectively). Second, MAb M9G1 does not react with lengthened peptides ^{22}Glu/Gly-Arg^{44} because the native structure of these peptides is changed as a result of enzymatic degradation of the protein. Third, the analysis of the PVM CP secondary structure indicates that the region of PVM CP at the position 34—49 is disposed to form the loop with π-turn (Fig. 2). Residues ^{39}Glu-Arg^{44} are involved in π-turn formation and residues at the positions 34—38 and 45—49 are flanked the π-turn at the N- and C-termini, respectively [21]. Hydrogen and hydrophobic interactions between the loop flanking residues support this formation at certain tension in the intact protein. The enzymatic destruction of CP, probably, results in the drastic distortions at the π-turn and flanking sites of the loop. It is likely such changes took place in tryptic peptides ^{22}Glu/Gly-Arg^{44} and abolished the recognition of M9G1 epitope by antibodies. However, these changes at the flanking parts of the loop did not affect the M6D5 epitope since most of epitope residues are located out of the loop (Fig. 3) and, therefore, all four tryptic peptides are recognised by MAb M6D5.

In order to confirm our assumption that MAbs M6D5 and M9G1 recognise two different epitopes and to determine relative positions of M4C1, M6D5 and M9G1 epitopes to each other, a dot immunobinding assay was performed. MAbs M6D5 and M9G1 as primary antibodies, MAb M4C1-HRP as a secondary antibody and CPs of U1, U7, U2, Russian strains as antigens and PVX as a negative control were used in this assay. It was found that MAb M6D5 did not block the interaction of MAb M4C1-HRP with PVM CPs, while MAb M9G1 blocked up the access of MAb M4C1-HRP to the all of PVM CPs (Fig. 4). Thus, it may be presumed that MAbs M4C1 and M9G1

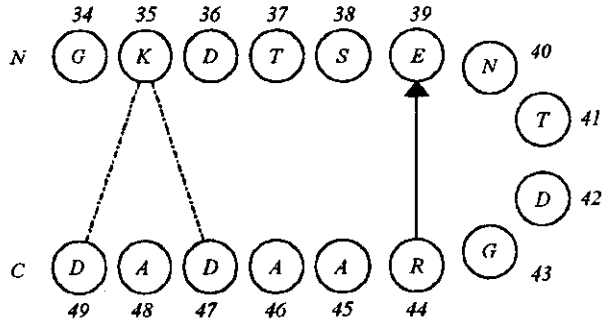

Fig. 2. Schematic representation of putative loop region with π-turn at the N-terminal part of PVM coat protein. Residues ^{34}Gly-Ser^{38} and ^{45}Ala-Asp^{49} flank the N- and C-termini of the π-turn, respectively. The hydrogen bond between N-H of Arg^{44} and C=O of Glu^{39} is shown by the arrow. Additional loop-stabilising hydrogen bond Lys^{35}-Asp^{47} (or Asp^{49}) is shown by dashed lines (– · – · –)

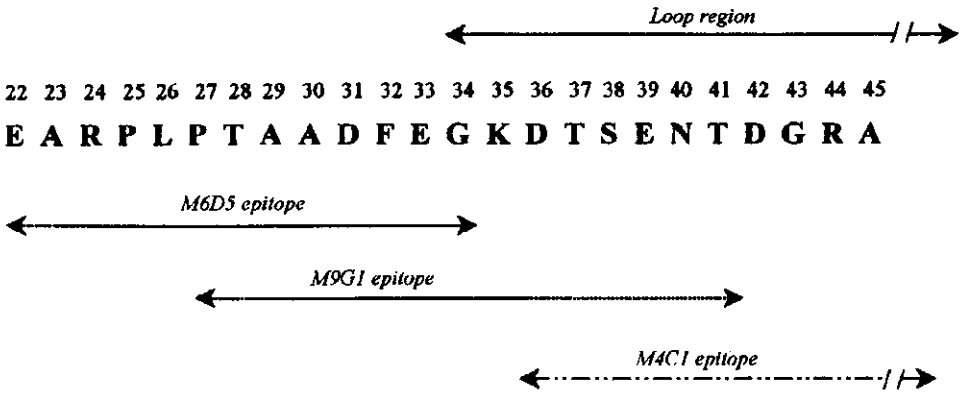

Fig. 3. Linear representation of relative locations of overlapping epitopes at the N-terminal region of PVM coat protein. Dotted lines (····) show the amino acid residues of epitopes which may contribute to the epitope-antibody interaction. Line (—···—) shows the putative position of M4C1 epitope

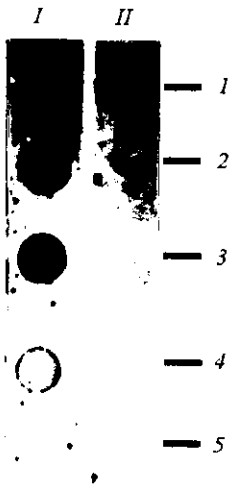

Fig. 4. Results of dot immunobinding assay. Nitrocellulose strips with adsorbed antigens were incubated with primary MAbs M6D5 (strip *I*) and M9G1 (strip *II*) and then incubated with secondary MAb M4C1-HRP. Antigens are presented in horizontal lines: *1* — PVM U1 coat protein (CP); *2* — PVM U7 CP; *3* — PVM Russian strain CP; *4* — PVM U2 CP; *5* — Potato Virus X CP (negative control)

recognise either closely located or overlapping, or conformational approximated epitopes.

To establish the contribution of Lys^{35} side chain in antibody-antigen interaction, tryptic peptides U1—14 and U7—16 were modified with citraconic anhydride and analysed by ELISA. It is known that the positive charge of amino acids containing free NH_2 groups is changed to a negative one by modification with citraconic anhydride. The results of this analysis (Fig. 5) showed that the reaction of MAb M6D5 with modified peptides was insignificantly decreased (less than 10 %), while the interaction between MAb M9G1 and the same peptides was approximately two-fold increased in comparison with non-modified peptides. This effect may be attributed to the presence of additional residues for M9G1 epitope that are situated out of C-termini of the used fragment. The change of lysine side chain positive charge to negative one may result in mimicry of the side chain negative charge of the aspartic acid residue (position 36), which is absent in the $^{22}Glu/Gly\text{-}Arg^{44}$ peptides. In addition, it may be suggested that Lys^{35} side chain positive charge does not contribute to the MAb M9G1-epitope M9G1 interaction, but, probably, takes part in forming of internal salt bridge with negatively charged side chain either Asp47, or Asp49 from the C-terminal flanking region of the loop (Fig. 2).

On the basis of epitope-mapping results the linear tetradecapeptide $^{22}EARPLPTAADFEGK^{35}$ (P14) was synthesised and its immunochemical analysis was carried out. In ELISA, MAbs M6D5 and M9G1 recognised the peptide P14 at concentration 1 pM per well (1.5 ng) and 100 pM per well (150 ng), respectively. The ability of peptide P14 to inhibit antibody-antigen interaction was evaluated in inhibition ELISA. It was shown that peptide P14 exhibited different inhibition activity against two MAbs (Fig. 6, 7). Peptide P14 inhibited reaction of MAb M6D5 with P14 (Fig. 6) or CP (Fig. 7, line *1*) in dose-dependent manner. In contrast, the inhibition of the reaction of MAb M9G1 with the same antigens did not show any linear dose-dependence. In the reaction of MAb M6D5 with antigens P14 (Fig. 6) and CP (Fig. 7, line *1*) the 50 % inhibition was reached at the inhibitor concentrations 25 pM and 2.5 nM,

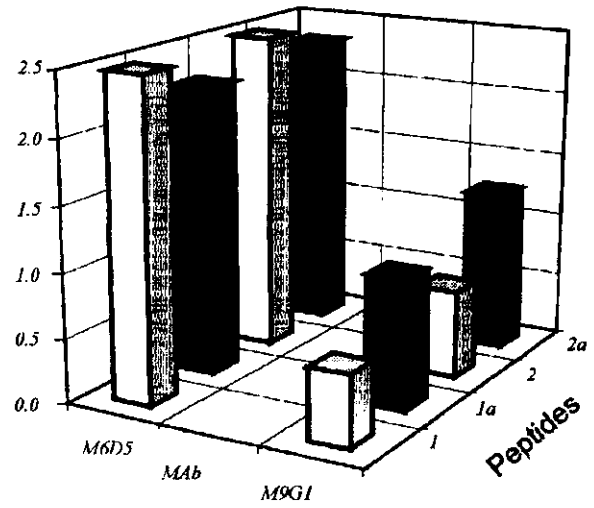

Fig. 5. Analysis of modified tryptic fragments ^{22}Glu/Gly-Lys^{35} with citraconic anhydride by ELISA using MAbs M6D5 and M9G1. Axis *y*: lines *1* and *2* — non-modified fragments ^{22}Glu-Lys^{35} of coat protein (CP) U1 and ^{22}Gly-Lys^{35} (CP U7), respectively; lines *1a* and *2a* — modified fragments ^{22}Glu-Lys^{35} (CP U1) and ^{22}Gly-Lys^{35} (CP U7), respectively

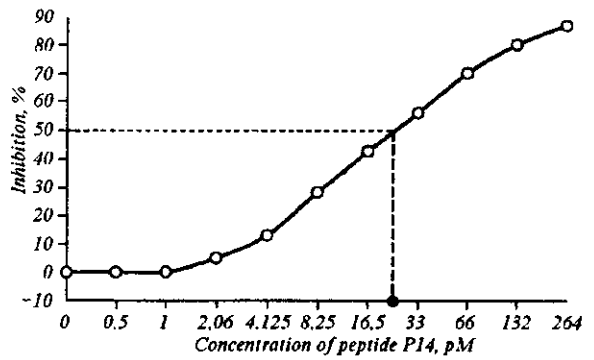

Fig. 6. Inhibition of the MAb M6D5-P14 interaction in ELISA by synthetic peptide P14. Concentration of coated P14 — 50 ng per well (33 pM). MAb M6D5 was used at the dilution 1 : 1000. Vertical dotted line (– – –) marks the peptide P14 concentration that is required for 50 % inhibition of antibody-antigen interaction. Positive reactions of MAbs with synthetic peptide ^{154}KDASSSVF^{161} used as negative control were not observed and these results are not shown

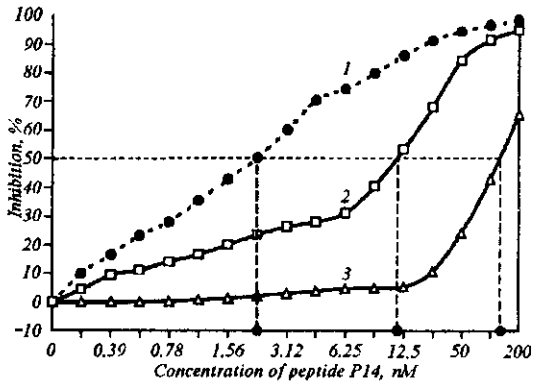

Fig. 7. Results of inhibition ELISA using synthetic peptide P14 as inhibitor. Lines: *1* — inhibition of MAb M6D5-CP (coat protein) U1 reaction; *2* — inhibition of MAb M9G1-P14 reaction; *3* — inhibition of MAb M9G1-CP U1 reaction. Coating concentrations of CP U1 — 500 ng (13 pM) per well, synthetic peptide P14 — 50 ng (33 pM) per well. MAbs were used at the next dilutions: M6D5 — 1 : 1000, M9G1 — 1 : 500. Vertical dotted lines (– – –) mark the peptide P14 concentrations that are required for 50 % inhibition of antibody-antigen interaction. Positive reactions of MAbs with synthetic peptide ^{154}KDASSSVF^{161} used as negative control were not observed and these results are not shown

respectively. Whereas for MAb M9G1 the same effects were observed at the inhibitor concentrations 11.8 nM and 128.6 nM (Fig. 7, lines *2* and *3*), respectively. The same concentrations of peptide P14 were more effective to inhibit interaction between MAb and P14 and less effective for MAb-CP interaction. The binding of MAb M6D5 with peptide P14 was inhibited at picomolar concentrations (Fig. 6), while the inhibition of MAb M6D5-CP, MAb M9G1-CP and MAb M9G1-P14 interactions was observed at nanomolar concentrations of peptide P14 (Fig. 7). The maximum inhibition by peptide P14 were as follows: 86.7 % for MAb M6D5-P14 interaction at inhibitor concentration (C_{inhib}) 264 pM, 98.3 % for MAb M6D5-CP, 95 % for MAb M9G1-P14 and 65.3 % for MAb M9G1-CP at the same C_{inhib} 200 nM in the last three interactions (Fig. 7). The inhibition ELISA results confirm our assumption that M9G1 epitope is partially represented in the tryptic fragments ^{22}Glu/Gly-Lys^{35} and synthetic peptide P14, while the main part residues of M6D5 epitope are, at least, located at the region ^{23}Ala-Gly^{34} of the PVM CP. Thus, the differences in the inhibition of antibody-antigen (PVM CP and peptide P14) interaction demonstrate the complex nature of the antigenic determinants, the importance of native conformation of antigen and epitope environment for antibody-antigen interaction.

In this study immunochemical analysis of the PVM CP antigenic structure has shown that the M6D5 and M9G1 epitopes are located at the N-terminal region of the PVM CP. Similar antigenic

structure of the filamentous viruses (potexviruses and potyviruses) with the localisation of the virus-specific epitopes at the N- and C-terminal regions of their CPs has been established earlier [22—24]. PVM-specific M6D5 and M9G1 epitopes overlap each other and have a common antibody-recognising site (within fragments ^{22}Glu/Gly-Lys^{35}). The M4C1 epitope is completely destroyed during proteolysis with trypsin and is either conformational approximated to, or overlapping with the M9G1 epitope (Fig. 3). Amino acid substitution at the position 22 (Glu → Gly) of the CP U7 does not affect the recognition of the two overlapping epitopes by antibodies. Modification of Lys^{35} side chain with citraconic anhydride increases interaction of MAb M9G1 with fragments ^{22}Glu/Gly-Lys^{35}. Taken together, the analysis of the antigenic structure of two PVM Ukrainian strains in present study and previous immunochemical characterisation of eighteen PVM strains [11] suggest a conservative organisation of PVM-specific epitopes at least for 20 PVM strains, isolated in five European regions. Nevertheless, it cannot rule out the existence of PVM strains with considerable changes in antigenic organization of coat protein subunit caused by natural and(or) human activity induced factors.

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С. С. Вітер, Т. Ю. Ткаченко, Л. П. Коломієць, Ю. Л. Радавський

Аналіз антигенної структури українських штамів М-вірусу картоплі

Резюме

Для аналізу антигенної структури білка оболонки двох українських штамів М-вірусу картоплі (PVM) U1 та U7 використано три моноклональних антитіла (МКА) проти PVM. Фрагменти ^{22}Glu-Lys^{35} та ^{22}Gly-Lys^{35}, одержані після ферментативного гідролізу трипсином білків оболонки двох штамів PVM, розпізнавалися двома МКА — M6D5 та M9G1. Заміна Glu → Gly в позиції 22 білка оболонки PVM U7 не змінювала розпізнавання антитілами як триптичних пептидів, так і білків оболонки цих штамів. МКА M9G1 та M4C1 конкурували між собою за ділянки зв'язування з молекулою білка оболонки PVM. Синтетичний пептид П14, який відповідав фрагментові ^{22}Glu-Lys^{35}, з різною ефективністю пригнічував взаємодію МКА M6D5 та M9G1 з фрагментом П14 і білком оболонки PVM. Зміна позитивного заряду бокової групи Lys^{35} на від'ємний шляхом модифікації пептидів ^{22}Glu-Lys^{35} та ^{22}Gly-Lys^{35} цитраконовим ангідридом призвела до дворазового посилення реакції МКА M9G1 та до незначного зниження реакції МКА M6D5 з модифікованим пептидом. На підставі одержаних результатів встановлено, що PVM-специфічні епітопи розташовані в N-кінцевій області білка оболонки. Два епітопи, які розпізнаються МКА M6D5 та M9G1, перекриваються та мають загальну ділянку зв'язування у фрагменті ^{22}Glu/Gly-Lys^{35}. Епітопи, які розпізнаються МКА M4C1 та M9G1, або перекриваються між собою, або конформаційно наближені один до одного.

С. С. Витер, Т. Ю. Ткаченко, Л. П. Коломиец, Ю. Л. Радавский

Анализ антигенной структуры украинских штаммов М-вируса картофеля

Резюме

Для анализа антигенной структуры белка оболочки двух украинских штаммов М-вируса картофеля (PVM) U1 и U7 использованы три моноклональных антитела (МКА) против PVM. Фрагменты ^{22}Glu-Lys^{35} и ^{22}Gly-Lys^{35}, полученные после ферментативного гидролиза трипсином белков оболочки двух штаммов PVM, распознавались двумя МКА — M6D5 и M9G1. Замена Glu → Gly в позиции 22 белка оболочки PVM U7 не изменяла распознавания антителами как триптических пептидов, так и белков оболочки этих штаммов. МКА M9G1 и M4C1 конкурировали между собой за участки связывания с молекулой белка оболочки PVM. Синтетический пептид П14, соответствующий фрагменту ^{22}Glu-Lys^{35}, с различной эффективностью ингибировал взаимодействие МКА M6D5 и M9G1 с фрагментом П14 и белком оболочки PVM. Изменение положительного заряда боковой группы Lys^{35} на отрицательный путем модификации пептидов ^{22}Glu-Lys^{35} и ^{22}Gly-Lys^{35} цитраконовым ангидридом привело к двухкратному усилению реакции МКА M9G1 и незначительному снижению реакции МКА M6D5 с модифицированным пептидом. На основании полученных результатов установлено, что PVM-специфические эпитопы расположены в N-концевой области белка оболочки. Два эпитопа, распознаваемые МКА M6D5 и M9G1, перекрываются между собой и имеют общий участок связывания во фрагменте ^{22}Glu/Gly-Lys^{35}. Эпитопы, распознаваемые МКА M4C1 и M9G1, либо перекрываются между собой, либо конформационно приближены друг к другу.

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