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IMMUNOCHEMICAL ANALYSIS OF THE SURFACE PROTEIN OF PLANT AND ANIMAL RHABDOVIRUSES

T.YU. SABIROVA, L.F. DIDENKO, N.YA. SPIVAK

Zabolotny Institute of microbiology and virology, National Academy of Sciences, Ukraine, Kiev, Zabolotny str.154, 03187, e-mail: Tatiana.Sabirova@gmail.com

Immunochemical study on antigenic relationship between surface proteins of different rhabdoviruses was carried out. Potato curly dwarf virus (PCDV), potato yellow dwarf virus (PYDV) and spot sweetflag virus (SSV) were studied as plant viruses and vesicular stomatitis virus (VSV) and rabies virus (RV) were studied as animals and humans rhabdoviruses. It was established on the base of experimental data using monoclonal serum to a surface protein PYDV that the rhabdoviruses under consideration do not have antigenic relationship of surface proteins.

Keywords: rhabdovirus, viral surface proteins, antigenic relationship

Introduction. Development of highly sensitive and specific detection methods for diagnostics of rhabdoviruses and effective measures to combat them requires a detailed study of their biological, structural and functional characteristics. Valuable information about the structural components of rhabdoviruses can be obtained by studying their antigenic properties. Rhabdoviral surface protein (G protein) is of particular interest, because on the fact that it is glycosylated and involved in the processes of adhesion (Coll, 1995; Da Poian et al., 2005).

Properties of rhabdoviral glycoprotein are largely determined by its carbohydrate component, which plays an important role in its structural organization. When comparing the monosaccharide composition of PCDV, SSV and VSV it was revealed that they contain mostly identical monosaccharides, which differ in the quantitative ratio (Didenko et al., 2008). It is important, that in the reaction immunodiffusion in gel polyvalent serum to PCDV reacted with SSV, VSV and RV.

It is known that carbohydrates have often antigenic activity. They are multivalent and therefore complementary antibodies are generated to specific structural sites. As part of PCDV, SSV, VSV and RV, mannose and glucose among other monosaccharides were found. Moreover, we proved the existence of structural analogy between immunodominant epitopes of rhabdoviral plant and animal carbohydrate components (Didenko et al., 2008). It appears from this that the structural components of the glycoprotein, namely carbohydrate, have antigenic activity and serological relatedness of rhabdovirus plants and animals.

On the base of the above, it is interesting to investigate the protein portion of a glycoprotein on its antigenic relatedness rhabdovirus PCDV, SSV, VSV and RV. To this end, we used monoclonal serum to G proteins potato yellow dwarf virus.

Materials and methods. To isolate phytorhabdoviruses tobacco plants (*Nicotiana rustica*) previously infected by PCDV and SSV were used. For the mechanical damage of leaves celite type 535 ("Serva") was used. Virus isolation was performed as follows: leaves of tobacco (*Nicotiana rustica*) with pronounced symptoms were homogenized in liquid nitrogen. Homogenate was added to a buffer that contained 0.1 M glycine, pH 8.0, 0.01 M MgCl2, 0,01 M Na2SO3. Solution was then filtered and the filtrate was centrifuged at 5000g for 10 minutes. From the resulting supernatant virus was deposited by 7% polyethylene glycol (PEG-6000) in the presence of 0.15 M NaCl. Precipitated virus was collected by centrifugation at 10000 g for 15 minutes. Sediment was resuspended in buffer solution containing 0.1 M glycine, 0.01 M MgCl2 at pH 7.0. For release from the PEG centrifugation was performed by at 10000g for 15 minutes. The virus was pelleted by centrifugation through 1.5 M sucrose cushion for 3 hours at 24000 rpm in rotor SW - 27 (Beckman) (Didenko et al., 2001).

Vesicular stomatitis virus (VSV) was cultured in continuous cell cultures of pig testicles. It was isolated according to previously described method (Dalton, Rose, 1993).

We used a vaccine strain of fixed rabies virus. The vaccine was a culture of industrial fixed-RV strain “Vnukovo-32", 30^{th} - 38^{th} passage, grown in

the kidney cells of Syrian hamsters, concentrated and purified by ultrafiltration or ultracentrifugation, inactivated by ultraviolet rays and formalin (Selimov et al., 1977). Vaccine was obtained from RV Enterprise for production of bacterial and viral agents of the Institute of Poliomyelitis and Viral Encephalitis M.N. Chumakov RAMS.

To carry out the immunochemical tests a polyvalent serum specific for PCDV, SSV, VSV and RV, obtained by intra-muscular immunization of rabbits with a mixture of equal volumes of purified virus preparation and Freund's complete adjuvant ("Calbiochem") was used. Rabbits were injected subcutaneously with 1.3 mg antigen in 0.5 ml. On the whole cycle of immunization 9 mg of antigen was used. After a month blood was collected and IgG was received by the Fogt method (Fogt, 1972). Also a commercial monoclonal serum (IgG) to the surface protein PYDV (DSMZ - Plant Virus Collection c\o Biologische Bundesanstalt Institut fur Pflanzenvirologie, Mikrobiologie und Biologische Sicherheit Messeweg 11\12 38104 Braunschweig Germany) was used. Normal serum was used as a control of uninfected rabbits.

Enzyme immunoassay was performed by indirect variant (Clark, Engvall, 1981; Kuate et al., 2006).

To sensitize the surface of the wells of polystyrene microplates in 0.1 ml of PCDV, SSV, VSV and RV preparations were used at a concentration of 5 mg/ml, diluted by 0.01 M Tris-HCl buffer pH 7,2-7,4 with 0,1 M NaCl. Antigen immobilization was performed during 16 hours at +4°C. The unbound antigen was washed with 1 M Tris-HCl, pH 7.38. Blocking of free binding sites on the plates was crried out by 0.5% solution of BSA in phosphate buffer, pH 7.6 with 0.05% Tween-20. To the wells with adsorbed antigen to 0.1 ml of monoclonal serum to PYDV and specific sera (positive control) at dilutions from 1:2 to 1:256 were added. Normal rabbit serum served as a control. For the quantitative determination of antibodies in the sera they were titrated by double dilution to 1 M Tris-HCl. They were thoroughly mixed for 7 seconds and incubated in a thermal 1,5 hours at +37°C. Then the plates were washed five times from unbound antibodies.

For qualitative estimation of the reaction (response "yes" or "no") setting was performed similarly, but sera were not titrated. 0.1 ml of conjugate (namely protein A *Staphylococcus aureus* labeled with peroxidase in a dilution 1/200000) was added to the washed wells. Microtiter wells were incubated for 1.5 hours at +37°C and washed. Then 0.1 ml of substrate mixture (0.15% solution of diaminobenzidine with 0.03% hydrogen peroxide) was added. After staining the product of the enzymatic reaction after 60 minutes the reaction was stopped by introducing into every well 0.05 ml 5 N HCl and absorbance was measured at grade reader Labsystems Multiskan MS at a wavelength of 450 nm.

Statistical analysis was linked immunosorbent assay was carried out taking into account the standard deviation (Lakin, 1980).

Results and discussion. For today antigenic properties of rhabdoviruses, despite their wide spread are still poorly understood. Convenient methods for quick and accurate diagnosis of rhabdovirus are not still developed. Analysis of antigenic structure of individual rhabdovirus is a necessary step towards the creation of immunochemical test systems for detecting and identifying them, in particular plant rhabdovirus: potato curly dwarf virus (PCDV), potato yellow dwarf virus (PYDV) and spot sweetflag virus (SSV) and well as human and animal rhabdovirus: vesicular stomatitis virus (VSV) and rabies virus (RV), which can provide information regarding features of the spatial organization of the subunits of structural proteins and viral particles as a whole. A study of antigenic relationship between rhabdovirus PYDV and related rhabdoviruses PCDV, SSV, VSV and RV by indirect Enzyme-Linked Immunosorbent Assay (ELISA) using commercial monoclonal serum to a surface protein PYDV was performed.

The following results were obtained by the ELISA: positive control specific polyvalent serum to viruses PCDV, SSV, VSV and RV had high optical density, indicating a high degree of specific binding of antigen with specific antibody. Negative control – normal rabbit serum did not react with any of the viruses we studied (Fig. 1).

ELISA analysis showed a negative level of fixation of viral proteins PCDV, SSV, VSV and the RV with monoclonal antibodies to surface protein PYDV. This may be due to the fact that on the surface of the virus represented only part of the epitope recognized by a monoclonal antibody to PYDV, and (or) that the conformation of the protein is not optimal for recognition by monoclonal antibody. It is true in this case we can not exclude the binding of polyvalent serum, including immunoglobulins to carbohydrates with surface antigens of rhabdovirus of plants and animals.

Thus, using the ELISA we no found antigenic relatedness of surface protein PYDV viruses PCDV, SSV, VSV and RV. As a result of these studies it can be suggested that it is the internal nucleocapsid proteins, and also, possibly, the carbohydrate portion of the surface proteins that exhibit antigenic relationship rhabdovirus (Didenko et al., 2008; Mandrika, 2007).

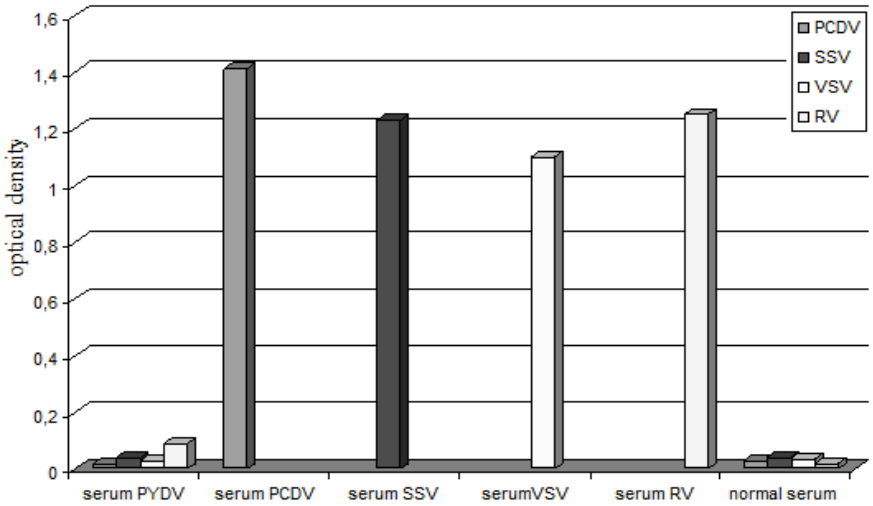

Fig. 1. The results of the ELISA

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ІМУНОХІМІЧНИЙ АНАЛІЗ ПОВЕРХНЕВОГО БІЛКА РАБДОВІРУСІВ РОСЛИН І ТВАРИН

Т.Ю. Сабірова, Л.Ф. Діденко, М.Я. Співак

Проведені імунохімічні дослідження щодо антигенної спорідненості поверхневих білків рабдовірусів рослин: вірусу кучерявої карликовості картоплі (ВККК), вірусу жовтої карликовості картоплі (ВЖКК) і вірусу плямистості аїру (ВПА), а також рабдовірусів тварин і людини: вірусу везикулярного стоматиту (ВВС) і вірусу сказу (ВС). На основі експериментальних даних при використанні моноклональної сироватки до поверхневого білку ВЖКК, встановлено, що досліджувані рабдовіруси не мають антигенної спорідненості поверхневих білків.

Ключові слова: рабдовірус, вірусний поверхневий білок, антигенні взаємодії.

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