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MONOSACCHARIDE COMPOSITION OF RHABDOVIRUSES INFECTING ANIMALS AND PLANTS

Comparative studies of plant and animal rhabdoviruses are of great importance because of their similar structural organization, morpholoical characters and functional properties of their components. Rhabdoviruses differ from plus-genome viruses, because in addition to their minus-RNA chain and multifunctional proteins, they contain also fatty acids and carbohydrates.

K e y w o r d s: carbohydrates, rhabdoviruses, vesicular stomatitis virus, curly potato dwarf virus, spot sweetflag virus.

Carbohydrate component of viral glycoproteins plays the leading role in the virus genome expression. Rhabdoviruses contain 3 % of carbohydrates. They are presented by N-glycan chains on the surface G-protein as well as by glycolipids (1, 2). G-protein function due to its association with specific monosaccharides determines to certain degree its properties; the most important G-protein property is peplomer formation on the virion surface, the peplomers being, in their turn responsible for virus binding to its host cell receptors (3). Besides, the carbohydrate roles in virus life cycle includes the glycoprotein transport to the cell surface and its intracellular migration (4), as well as carbohydrate participation in the formation and establishment of glycoproteins conformation necessary to assure their immunological properties; carbohydrates defend glycoprotein polypeptide chains against their non-specific cleavage by cell proteinases (5). Many laboratories investigate the different properties and the role of glycoproteins contained by vertebrates (6, 7) and plants (8).

The importance of such investigations is without any doubt because of increasing interest in biological evolution and properties of Rhabdoviridae family members infecting plants and animals. Taking into account these considerations, we have carried out the comparative study concerning detection and identification of carbohydrates contained by phytorhabdoviruses — curly potato dwarf virus (CPDV) and spot sweetflag virus (SSV), as well as by an animal rhabdovirus — causative agent of vesicular stomatitis (vesicular stomatitis virus, VSV).

Materials and methods

Isolation of viruses. Phytorhabdoviruses — curly potato dwarf virus (CPDV) and spot sweetflag virus (SSV) — were isolated from infected Nicotiana rustica tissues using PEG-6000 and differential centrifugation (7).

VSV was cultivated in the cells of embryonal piglet testicles; a purified virus preparation was obtained according to Dalton and Rose (8).

Carbohydrate identification. To identify neutral monosaccharides, the virus preparations were hydrolyzed by 2 N HCl during 5 h at 100 °C and then analyzed as polyol-acetates in a chromatomass-spectrometric Agilent 6890N/5973 inert system. A column PB225mS used had the parameters 30 m x 0.25 mm x 0.25 μm; the carrier gas, helium, was flowed through the column (1 ml/μm). The temperatures were 250 °C, 280 °C, and 22 °C for evaporator, interface, and thermostat, respectively, the process having been carried out in isothermal conditions.

The test was conducted by the division of the stream 1:100. The identification of monosaccharides was made by comparing of retention time for different polyol-acetates of samples tested and standard ones using the computer data base Chemstation. The quantitative ratios of individual monosaccharides were determined as percents from the amount of the peak areas of all monosaccharides.

To evaluate the aminosugar content, 1 mg of preparation was hydrolyzed by 6 N HCl (20 h, 100 °C). A hydrolyzate obtained was centrifuged and vacuum-evaporated. Aminosugars were determined by using of the amino acid analyzer KLA-5 ("Hitachi", Japan) using a column (0.9Ч15cm) containing "Ostion 0803" cation-exchanger in the sodium-citrate buffer, pH 5.28, at 55 °C. The quantitative glucosamine and galactosamine determination was carried out following the sample hydrolysis by 2 N CF_3CO_2H (1.5h, 120 °C) using Agilent 6890N/5973 inert system.

Results and discussion

The qualitative and quantitative monosaccharide contents in glycoproteins of viruses grown in animal cells, VSV, and plant ones — CPDV and SSV have been investigated.

The envelope structures of all three rhabdoviruses CPDV, SSV, and VSV were shown to contain monosaccharides, their contents being varied depending on the virus studied (Table 1).

Identifying monosaccharides contained by the CPDV we found out glucose (35.2 %) and mannose (23.8 %) to be dominant CPDV monosaccharides. In addition, this virus contains also galactose, arabinose, fucose, and rhamnose.

The carbohydrate component of the SSV was shown to include something different monosaccharide content. Its monosaccharides were glucose (25,3 %), galactose (18,3 %), arabinose (16 %), rhamnose (3,1 %), mannose (2.32 %), and fucose (3,98 %).

Sometimes we found out also ribose in several hydrolyzate preparations, its detection being due to the ribose presence in rhabdoviral RNA molecules.

Rhabdoviruses possess a very wide host range, their surface glycoproteins include carbohydrates of different cell origin leading to marked differences in monosaccharide contents of these pathogenic agents. That is why it is interesting to compare carbohydrate compounds included into CPDV and SSV particles, these agents being

reproduced in the same host, Nicotiana rustica. CPDV and SSV were shown to contain identical monosaccharides, their quantitative ratios being, however, markedly different.

The results obtained show the highest glucose content (35,2 %) is found for the CPDV. Contrary to the CDPV, the SSV contains lower quantity of this compound — 25,3 %.

The quantities of arabinose found in the CPDV and SSV are 10,4 % and 16 %, respectively. Phytorhabdoviruses contain also rhamnose, its quantities being 3,1 % and 9,7 % for the SSV and CPDV, respectively. Mannose is a dominating CPDV monosaccharide (23,8 %), its content in the SSV is significantly lower (2,32 %).

Besides, fucose was also identified in these viruses, its quantities being 8,6 % and 3,98 % for the CPDV and SSV, respectively.

Galactose levels in these agents are 12,3 % (in CPDV virions) and 18,3 % (in SSV particles). The presence of identical carbohydrates in phytorhabdoviruses infecting the same host demonstrates the viral carbohydrate structures to be predetermined by the host cell enzymatic systems (11). However, there is a point of view the virus is able to transform in some way the host cell glycosyltransferases (12). It is quite possible the cell glycosyltransferases modified by the viral infection may induce some changes concerning monosaccharide quantitative ratios in the cells and, consequently, in the virus particles.

Comparing monosaccharide contents of the VSV (an animal virus) and phytorhabdoviruses, CPDV and SSV, it was detected, the VSV contained much higher quantities of the mannose — 58,5 %; it is almost twice as much as for the CPDF (23,8 %) and about by 20 times more than in the SSV (2,32 %). The glucose contents are almost similar for the VSV (21 %) and for the SSV (25,3 %); VSV, however, contain less glucose quantity than the CPDV (35,2 %). The SSV contains also higher quantities of galactose 18,3 % and arabinose 16 % comparing to the CDPV, the last includes 12,3 and 10,4 % of these compounds, respectively. The fucose content of the VSV (3,7 %) is the same to the SSV (3,98 %), being, however, lower than in the CDPV (8,6 %). It is noteworthy there is no rhamnose in the VSV envelope, the SSV containing 3,1 % and the CDPV — 9,7 % of this monosaccharide.

Table 1

Comparative analyses of viral monosaccharides (%)

Monosaccharides	CPDV	SSV	VSV
Glucose	35,2	25,3	21,0
Mannose	23,8	2,32	58,5
Galactose	12,3	18,3	3,8
Arabinose	10,4	16,0	2,7
Fucose	8,6	3,98	3,7
Rhamnose	9,7	3,1	

All the viruses studied here include also, in addition to neutral monosaccharides, two aminosugars — glucosamine and galactosamine. The last compound content was markedly higher comparing to the first one: it was by 8,75 times for the CPDV, by 7,2 times for the SSV, and by 1,6 times more for the VSV.

REFERENCES

1. Murphy F.A., Fanquet C.M., Bishop D.V.L., Ghabrial S.H., Jarvis A.W., Mantelli G.P., Mayo M.A., Summers M.D. (eds) — Virus Taxonomy Classification and Nomenclature of Viruses. Sixth Report of International Committee on Taxonomy of Viruses // Virology. Division International Union of Microbiological Societies: Springer Verlag, Wien New York. — Arch. Virol. — 1995. — Suppl. 10. — 275-288.
2. Jackson A. O., Dietzgen R.G., Geodin J.M., Bragg J.N., Deng M. Biology of plant rhabdoviruses. // Ann. Rev. Phytopathol.— 2005.— V.43, p. 623-660.
3. Da Poian A.T., Carneito F.A., Stauber F. Viral membrane fusion: is glycoprotein G of Rhabdoviruses a representative of a new class of viral fusion proteins? // Brazilian Journal of Medical and Biological Research.— 2005.— V. 38, p. 813-823.
4. Machamer C.F., Gnar J.-L., Rose J.K. Role of glycolsylation in protein transport to the cell surface. — Virus Res., 1985, 3, Suppl. №1.
5. Derevitskaya V.A. Glycoproteins of RNA-containing enveloped viruses. Bioorganic Chemistry. Rus. — 1983. — V.9, №5, 581—616.
6. Coll J.M. The glycoprotein G of rhabdoviruses. // Arch. Virology.— 1995.— V. 140, p. 827-851.
7. Walker P.J., Kongsuwan K. Deduced structural model for animal rhabdovirus glycoproteins. // J.Gen.Virol. — 1999.— V. 80, p. 1211-1220.
8. Goldberg K.-B., Modrell B., Hillman B.I. Heaten L.A., Choi T-J., Jakson A.O. Structure of the glycoprotein gene of sonchus yellow net virus, a plant rhabdoviruses. // Virology.— 1991.— V. 185, p. 32-38.
9. L.F. Didenko, N.I. Parhomenko, L.O. Maksimenko, N.S. Dyachenco, N.M. Zaritsky, F.E Kozar. Influence of klinostation on the virus of curly potato dwarf virus (CPDV) and his structural components in vitro and in vivo. // Space science and technology— 1999.—5, p. 1-5.
10. Dalton K.P., Rose J.K. Vesicular stomatitis virus glycoprotein containing the entire green fluorescent protein on its cytoplasmic domain is incorporated efficiently into virus particles. // Virology.— 2001.—V. 279, №2, 414-421.
11. Zaides V.M., Berezin V.E., Zhdanov V.M. Glycoproteins of enveloped animal viruses as transmembrane proteins. Problems of Virology, 1986, vol. 31.— №2,—p. 133-148.
12. Klenk H.D. The molecular basis of microbiology. Patogenecity // Eds by H. Smith, J. Skeher, M. Turner. — Welnheim, 1980, 55-66.

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МОНОЦУКРИДНИЙ СКЛАД РАБДОВІРУСІВ, ІЗОЛЬОВАНИХ З ТВАРИН ТА РОСЛИН

Реферат

Вивчений моноцукридний склад глікопротеїнів зоорабдовіруса везикулярного стоматиту (ВВС) і фітопатогенних рабдовірусів — віруса кучерявої карликовості картоплі (ВККК) і віруса плямистості аїру (ВПА). При порівнянні моноцукридного складу всіх трьох представників рабдовірусів виявлені загальні моноцукриди — глюкоза, маноза, галактоза, арабіноза, фукоза. Домінуючими з них в складі ВККК і ВВС присутні глюкоза і маноза (35,2 %,

21,6 % і 23,8 %, 49,6 % відповідно), для ВПА глюкоза і галактоза (24 % і 16,4 % відповідно). ВКРК і ВПА на відміну від ВВС містять рамнозу (3 % і 9,7 % відповідно).
У всіх цих вірусах поряд з моноцукрами виявлені також і аміноцукри глюкозамін і галактозамін.

К л ю ч о в і с л о в а: вуглеводи, рабдовіруси, везикулярний вірус стоматиту, вірус кучерявої карликовості картоплі, вірус крапчатості аїру.

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МОНОСАХАРИДНЫЙ СОСТАВ РАБДОВИРУСОВ, ИЗОЛИРОВАННЫХ ИЗ РАСТЕНИЙ И ЖИВОТНЫХ

Реферат

Исследован моносахаридный состав гликопротеинов зоорабдовируса везикулярного стоматита (ВВС) и фитопатогенных рабдовирусов вируса курчавой карликовости картофеля (ВКРК) и вируса крапчатости аира (ВКА). При сравнении моносахаридного состава всех трех представителей рабдовирусов выявлены общие моносахариды — глюкоза, манноза, галактоза, арабиноза, фукоза. Доминирующими среди них в составе ВКРК и ВВС присутствует глюкоза и манноза (35,2 %, 21,6 % и 23,8 %, 49,6 % соответственно), для ВКА глюкоза и галактоза (24 % и 16,4 % соответственно). ВКРК и ВКА в отличие от ВВС содержат рамнозу (3 % и 9,7 % соответственно).
Во всех этих вирусах, наряду с моносахарами, выявлены также и аминосахара — глюкозамин и галактозамин.

К л ю ч е в ы е с л о в а: углеводы, рабдовирусы, везикулярный вирус стоматита, вирус курчавой карликовости картофеля, вирус крапчатости аира.