

Characterization and Location of Sowthistle Yellow Vein Virus Proteins.

M.IEMIECKI

*Corresponding author

M. IEMIECKI,
Department of Virology, Agricultural University, Binnenhaven 1 t, Wageningen, The Netherlands.

Received Date : May 12, 2024

Accepted Date : May 14, 2024

Published Date : June 14, 2024

INTRODUCTION

Sowthistle yellow vein virus (SYVV) is an enveloped bacilliform virus that infects sowthistle (*Sonchus oleraceus* L.) and lettuce (*Lactuca sativa* L.). It is spread persistently by the aphid *Hyperomyzus lactucae* L. Vein banding and vein clearing are indicators of infection in these plants (Duffus, 1963; Richardson & Sylvester, 1968 ; Peters, 1970).

SYVV has been assigned to the rhabdovirus group based on the morphological and physicochemical data that are currently known.

The shape of rhabdoviruses that infect plant and vertebrate cells varies both in situ and in vitro, as well as in terms of where in the cell they develop and envelope (Peters & Schultz, 1975). Comparative research on the structural proteins of the rhabdoviruses infecting the two types of cells is necessary in light of these distinctions.

Similarly, information regarding the location of rhabdovirus structural proteins has been derived nearly solely from viruses that infect vertebrates, most notably rabies and VSV. According to various theories (Wagner et al., 1972; Knudson, 1973; Emerson & Yu, 1975; Imblum & Wagner, 1975), the G protein is connected to the surface projections, the N protein is the major nucleocapsid protein, the L and NS proteins are minor nucleocapsid proteins implicated in replicase activity, and the M protein or proteins are connected to the virus membrane. The location of the structural proteins of rhabdoviruses that infect plants is not well understood.

METHODS

Multiplication and purification of viruses. Sowthistle (*Sonchus oleraceus* L.) plants cultivated in a typical glasshouse were

used to propagate SYVV. Dr. J. E. Dougus kindly contributed the original viral isolation, which was used throughout. Four to five week old plants were inoculated with SYVV-infected aphids (*Hyperomyzus lactucae* L.). For virus purification, infected leaves exhibiting vein clearing symptoms (14 to 21 days post-infection) were employed. The virus was cleansed using Ziemiacki & Peters' (1976) modified version of Peters & Kitajama's (1970) technique.

Using bovine serum albumin as a reference, the concentration of virus in purified preparations was measured in terms of protein according to Lowry et al. (1951). electron microscopy. Prior to examination, all samples were fixed for 10 minutes with an equal volume of 2.0% glutaraldehyde in double distilled water. Grids were then floated on the water for 10 minutes to remove any excess glutaraldehyde. Finally, the samples were stained with either 2.0% phosphotungstic acid in double distilled water that had been pH-adjusted with NaOH or 2.0% unbuffered uranyl acetate. A Siemens Elmiskop 101 electron microscope was used to analyze the samples.

protein electrophoresis. Every sample used for the electrophoretic analysis was heated to 1% SDS and boiled for five minutes. Weber & Osborne (1969) described staining, destaining, and electrophoresis in cylindrical gels. Additionally, protein from the iodinated virus was electrophoresed utilizing the discontinuous buffer system of Laemmli (1970), 3% stacking gel, and 11% resolving gel, using a slab gel apparatus (Studier, 1973). For 5-8 hours, the electrophoresis was run at 50 V. Slab gels were stained in 50% methanol and 7% acetic acid with 0.25% Coomassie brilliant blue G 250 (Merck) and then destained in the same mixture. Vacuum was used to dry stained gels on Whatman 3MM filter paper, basically as instructed by Maizel (1971). The gels were soaked for 1 h in a solution of 5% glycerol and 50% methanol before drying. For autoradiograms, Kodak medical X-ray film (RP Royal, X-Omat) was utilized.

Therapy with enzymes. The following enzymes were tested for their impact on protein composition and particle shape. The variables that are stated in brackets are the temperature, time of incubation, source, and buffer used. Pronase (B grade, Calbiochem, 0.025 M-tris/HCl, pH 7.3, 37 °C, 0 to 60 min), bromelain (Sigma, 0.1 M-citrate, pH 4.5, or 0.1 M-tris/HCl, pH 7.2, 37 °C, 0 to 60 min), and trypsin (Sigma, 0.025 M-tris/HCl, pH 7.6, 37 °C, 0 to 3 h), thermolysin (Sigma, 0.1 M-tris/HCl, pH 7.6, containing 0.1 M-NaCl, 0.005 M-CaCl₂, 37 °C, 0 to 60 min). The virus that had been enzyme-treated was seen under an electron microscope, and if needed, it was immediately dissociated using SDS and electrophoresed. Prior

to dissociation, enzyme activity was suppressed in all tests using thermolysin and in some assays using trypsin.

Iodination of both undamaged and whole viruses. Before iodination, the virus was disrupted by a 30-minute room temperature incubation with 0.1% Nonidet P40. The mixture used in the process for the enzymatic iodination process was catalyzed by lactoperoxidase and involved 1.6 ml of buffer (GMA I buffer, 1% strength GMA I, or 0.05 M-tris/HCl, pH 7.5) with 20 #l 0.1 mM-potassium iodide, 25 #l 0.25 mM hydrogen peroxide (H₂O₂) and 25 #l carrier free ¹²⁵I, Amersham Radiochemical Centre, U.K.; 50 #g intact or disrupted virus. Depending on the ratio of ¹²⁵I to virus (1/~Ci ¹²⁵I/~g SYVV = 0.001 to 0.33), different amounts of ¹²⁵I were employed. Three 25/~1 samples of 0.25 mM-H₂O₂ were added to the reaction at two-minute intervals after a gentle agitation and the addition of 25 #l lactoperoxidase solution (E250—0.08). When desired, the reaction was stopped by adding 0.5 ml of L-cysteine hydrochloride and letting it cool on ice. A 5% sucrose cushion was used to sediment the intact tagged virus (40000 rev/min in a SW 50.1 rotor). The pellet was then resuspended in 0.1 ml 0.1%.

RESULTS

After the purified virus was electrophoresed on 5, 7.5, and 10% acrylamide gels, four major and one minor protein were seen. The protein band patterns were unaffected by the addition of 2-mercaptoethanol to the disruption and electrophoresis buffers. The densitometer pattern of isolated viral proteins electrophoresed on a 7.5% acrylamide gel is displayed in Figure 1, trace (a). According to Wagner et al.'s proposal (1972), the structural proteins' nomenclature has been adopted. The structural proteins' estimated weights, as determined by 10% acrylamide gel calculations, were 150000 (high molecular weight protein), 83000 (G), 60000 (N), 44000 (M1), and 36000 (M2). The minor high weight protein content differed depending on the preparation.

The 150000 molecular weight component and G protein had covalently attached carbohydrates, as determined by periodic acid-Schiff's staining (Fig. 1, trace b). Periodic acid-Schiff's reagent stained both adjacent bands that included G protein positively whenever they appeared, but no quantitative difference in staining intensity could be found. Mol. wt. measurements on various % acrylamide gels provided additional proof of the glycoprotein origin of the high mol. wt. component and G protein (Segrest & Jackson, 1972). While the values for the other proteins stayed constant, the measured tool weight of these proteins fell when the percentage of acrylamide in the gels (5, 7.5 and so on) increased.

Three experimental strategies were used: (1) preparing subviral structures with detergents and identifying the structural proteins they contained; (2) using proteolytic enzymes to eliminate proteins outside of the virus membrane; and (3) iodinating purified virus preparations both enzymatically and non-enzymatically. Electron microscopy was used in tandem with the first two methods.

Displays the electrophoretic patterns that were produced after the virus was treated with trypsin for various amounts of time. By promptly heating the reaction mixture to 100°C of SDS and boiling it, the reaction was stopped. The entire disturbed blend was administered onto the gels. The observed molecular weight (mol. wt.) of the G protein (gels 1 and 2) decreased quickly (by about 5 to 7000), and this was followed by a progressive decline in the quantity of the lower mol. wt. G protein. According to analyses of structural proteins (Figs. 4 and 5) and electron microscopy (Fig. 2), all of the projections and all of the G protein were entirely removed from the intact virus after a 25-minute trypsin treatment.

DISCUSSION

The Wagner et al. (1972) proposal is followed in the nomenclature of the SYVV proteins (Fig. 1); however, this is based on location and function as criterion. The nomenclature used here may need to change when additional information becomes available because of the difficulty in definitively determining the location and function of the SYVV proteins, particularly M1 and M2. For the structural proteins in this investigation, the tool weight values are comparable to those reported by Schultz & Harrap (1976), with the exception of G protein, which they discovered to be 71000. The same isolate of SYVV was used in our laboratory for subsequent measurements, which produced results comparable to those presented here (M. G. Schultz, personal communication).

The variation could be the result of variations in the virus's propagation environment or in the selection of the gel system and protein markers.

Rhabdoviruses can be classified as rabies-like and VSV-like viruses using the SDS-gels with protein patterns (Lenoir & De Kinkelin, 1975). Unlike PYDV, the electrophoretic patterns seen with purified SYVV place this virus in the rabies-like category. Its protein profile resembles that of VSV (Wagner, Schnaitman, & Snyder, 1969; Knudson & MacLeod, 1972). It is still to be determined if such a classification has any influence on morphology and biological characteristics.

More research is necessary to understand the circumstances surrounding the high molecular weight protein seen in pure SYVV preparations. Small quantities of a true L protein, which is described as a unique polypeptide encoded by the virus genome, linked to the nucleocapsid, and involved in replicase activity (Stampfer & Baltimore, 1973; Emerson & Yu, 1975;

Imblum & Wagner, 1975), may exist, but the information gathered on the SYVV high molecular weight protein does not support such a function. This protein appears to be a dimer of G protein, in our opinion. Our conclusion is supported by its approximate molecular weight, the presence of carbohydrates, the removal of the material using proteolytic enzymes, and the material's interaction with the membrane fraction after Nonidet P40 treatment. 0970 Sokol et al. noted Upon SDS/z mercaptoethanol disruption of the aH-glucosamine-labelled virus, an 160000 small component was observed on SDS-gels, and it was proposed that this component was a G protein dimer. The pure envelopes from VSV treated with saponin were shown to include the L protein (Arstila, 1974), which could be comparable to the high molecular weight protein found in SYVV. Previous research has shown that stable glycoprotein aggregates are generated following SDS disruption (Tuech & Morrison, 1974). When naming rhabdovirus structural proteins, it is important to keep in mind the observation of big proteins that are different from the L protein on SDS-gels.

Iodination of whole virus particles verified the G protein's exterior placement, while proteins Mt and M2 appear to be spatially intermediate between the nucleocapsid and the projection and are similarly iodination-accessible. This would imply a connection to the membrane, which explains the names given to these two proteins. The N protein's internal position corresponds with its late labeling. Although the high tool. wt. protein's labeling was delayed and suggested an internal position, this did not rule out the idea that the protein is a dimer of G, as some iodination of G protein was required before the high tool. wt. protein appeared labeled.

REFERENCES

1. ARSTILA, P. (1974). Characteristics of vesicular stomatitis virus envelopes released with saponin. *Journal of General Virology* 24, 319-326.
2. CARTWRIGHT, B., SMALE, C. J. & BROWN, F. (1970). Dissection of vesicular stomatitis virus into the infective ribonucleoprotein and immunizing components. *Journal of General Virology* 7, 19-32.
3. DURFUS, J. E. (1963). Possible multiplication in the aphid vector of sowthistle yellow vein virus, a virus with an extremely long insect latent period. *Virology* 21, 194-202.
4. EMERSON, S. U. & YU, Y.-H. (1975)- Both NS and L proteins are required for in vitro RNA synthesis by vesicular stomatitis virus. *Journal of Virology* 15, 1348-1356.
5. FRANCKI, R. I. a. & RANDL~, J. W. (1974). Composition of the plant rhabdovirus lettuce necrotic yellows virus in relation to its biological properties. In *Negative Strand Viruses*, pp. 224-242. Edited by B. W. J. Mahy & R. D. Barry. London: Academic Press.
6. GAHMBERG, C. G., UTERMANN, G. & SIMONS, K. (1972). The membrane proteins of Semliki Forest virus have a hydrophobic part attached to the viral membrane. *FEBS Letters* 28, 179-182.
7. HILL, B. J., UNDERWOOD, B. O., SMALL, C. J. & BROWN, F. (1975). Physicochemical and serological characterization of five rhabdoviruses infecting fish. *Journal of General Virology* 27, 369-378.
8. IMBLUM, R. L. & WAGNER, R. R. (1975). Inhibition of viral transcriptase by immunoglobulin directed against the nucleocapsid NS protein of vesicular stomatitis virus. *Journal of Virology* xS, 1357-1366.
9. KNUDSON, D. L. (1973). Rhabdoviruses. *Journal of General Virology* 20, Supplement, 105-130.
10. KNUDSON, D. L. & MACLEOD, R. (1972). The proteins of potato yellow dwarf virus. *Virology* 47, 285-295.
11. LAEMMLT, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, London* 227, 680-685.
12. LENOIR, G. & DE KINKELIN, P. (1975). Fish rhabdoviruses : comparative study of protein structure. *Journal of Virology* x6, 259-262.
13. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* x93, 265-275.
14. MAIZEL, J. V. 0970- Polyacrylamide gel electrophoresis of viral proteins. In *Methods in Virology*, vol. V, pp. 179-246. Edited by K. Maramorosch & H. Koprowski. New York, London: Academic Press.
15. MOORE, Y. F., KELLEY, J. M. & WAGNER, R. R. (1974). Envelope proteins of vesicular stomatitis virions : accessibility to iodination. *Virology* 61, 292-296.
16. MODD, J. A. (1974). Glycoprotein fragment associated with vesicular stomatitis virus after proteolytic digestion. *Virology* 62, 573-577.

17. PETERS, D. 0970. Sowthistle yellow vein virus. C.M.L/ A.A.B. Descriptions of Plant Viruses no. 62.
18. PETERS, D. & KITASIMA, E. W. 0970). Purification and electron microscopy of sowthistle yellow vein virus. Virology 4 x, 135-150.
19. PETERS, D. & SCHULTZ, i.G. 0975)- A model for rhabdovirus morphogenesis. Koninklijke Nederlandse Akademie van Wetenschappen, C, 78, 172-181.
20. HILLIPS, D.R. & MORRISON, M. 0970). Arrangement of proteins in the human erythrocyte membrane. Biochemical and Biophysical Research Communications 4 ° , 284-289. PmLLIPS, r). R. & MORRISON, M. (~971). Exposed protein or~ the intact human erythrocyte. Biochemistry xo, 1766-1771.
21. RICHARDSON, S. & SYLVESTER, E. S. (1968). Further evidence of multiplication of sowthistle yellow vein virus in its aphid vector, *Hyperomyzus laetueae*. Virology 35, 347-355.
22. SCHLESINGER, M. J., SCHLESINGER, S. & BURGE, a. W. (1972). Identification of a second glycoprotein in Sindbis virus. Virology 47, 539-541.
23. SCHLOEMER, R. H. & WAGNER, R. R. 0975). Association of vesicular stomatitis virus glycoprotein with virion membrane: characterization of the lipophilic tail fragment. Journal of Virology 16, 237-249.
24. SCHULTZ, i. G. & HARRAP, K. A. (1976). The structural polypeptides of sowthistle yellow vein virus (SYVV). Archives of Virology 50, 173-176.
25. SEGREST, J. P. & JACKSON, R. L. (1972). Molecular weight determination of glycoproteins by polyacrylamide gel electrophoresis in sodium dodecyl sulphate. In *Methods in Enzymology*, 28, part B, pp. 54-63. Edited by S. P. Colowick & N. O. Kaplan. New York, London: Academic Press.
26. SIMONS, K., KERNEN, S. & KÄMÄNEN, L. (1973). Identification of a precursor for one of the Semliki Forest virus membrane proteins. FEBS Letters 29, 87-91.
27. SOKOL, E., STANCEK, D. & KOPROWSKI, H. 0970. Structural proteins of rabies virus. Journal of Virology 7, 241-249.
28. STAMPEER, i. & BALTIMORE, D. (1973). Identification of vesicular stomatitis virus large protein as a unique viral protein. Journal of Virology xx, 520-526.
29. STANLEY, P. & HASLAM, E. A. (1971)- The polypeptides of influenza virus. V. Localization of polypeptides in the virion by iodination techniques. Virology 46, 764-773.
30. STUDIER, E. W. 0973)- Analysis of bacteriophage T7 early RNAs and proteins on slab gels. Journal of Molecular Biology 79, 237-248.
31. TSAI, C.-M., HUANG, C.-C. & CANELLAKIS, E. S. (1973). Iodination of cell membranes. I. Optimal conditions for the iodination of exposed membrane components. Biochimica et Biophysica Acta 332, 47-58.
32. TUECK, S. K. & MORRISON, M. 0974). Human erythrocyte membrane sialoglycoproteins: a study of interconversion. Biochemical and Biophysical Research Communications 59, 352-360.
33. WAGNER, R. R., PREVEC, L., BROWN, E., SUMMERS, D. E., SOKOL, F. & MACLEOD, R. (1972). Classification of rhabdovirus proteins: a proposal. Journal of Virology xo, 1228-1230.
34. WAGNER, R. R., SCHEIDT, Z. A. & SNYDER, R. M. 0969). Structural proteins of vesicular stomatitis virus. Journal of Virology 3, 395-403.
35. WALTER, G. & MUDD, S. A. 0973)- Iodination of vesicular stomatitis virus with lactoperoxidase. Virology 52, 574-577.
36. WEBER, K. & OSBORNE, M. 0969)- The reliability of molecular weight determinations by dodecyl sulfate polyacrylamide gel electrophoresis. Journal of Biological Chemistry 244, 4406-4412.
37. ZACHARIUS, R. M., ZELEVANSKY, V. E. & MORRISON, J. H. (1969). Glycoprotein staining following electrophoresis on acrylamide gels. Analytical Biochemistry 30, 148-152.
38. ZEMMECK, G. & IERS, O. 0976). Selective proteolytic activity associated with purified sowthistle yellow vein virus preparations. Journal of General Virology 3x, 373-381.