Research Article

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Characterization and Location of Sowthistle Yellow Vein Virus Proteins.

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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 I-lyperomyzus lactucae L. Vein banding and vein clearing are indicators of infection in these plants (Duffus, I963; Richardson & Sylvester, I968 ; Peters, I970).

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, I975). 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., I972; Knudson, I973; Emerson & Yu, I975; Imblum & Wagner, I975), 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 Ziemiecki & Peters' (r976) modified version of Peters & Kitajama's (I97o) 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. 095I). electron microscopy. Prior to examination, all samples were fixed for 10 minutes with an equal volume of 2 oo 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 oo phosphotungstic acid in double distilled water that had been pH-adjusted with NaOH or 2 oo unbuffered uranyl acetate. A Siemens Elmiskop Ioi 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 (I969) described staining, destaining, and electrophoresis in cylindrical gels. Additionally, protein from the iodinated virus was electrophoresed utilizing the discontinuous buffer system of Laemmli (I97o), 3 ~ stacking gel, and 11 ~ resolving gel, using a slab gel apparatus (Studier, r973). For 5-8 hours, the electrophoresis was run at 50 V. Slab gels were stained in 50 methanol and 7 acetic acid with o'~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 (I97I). The gels were soaked for I h in a solution of 5 ~ glycerol and 5 o/ /o 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, o.o25 M-tris/HCl, pH 7"3, 37 °C, o to 6o min), bromelain (Sigma, o.I M-citrate, pH 4"5, or o.I M-tris/HC1, pH 7.2, 37 °C, o to 6o min), and trypsin (Sigma, o-o25 M-tris/HC1, pH 7.6, 37 °C, o to 3 h), thermolysin (Sigma, o.I M-tris]HC1, pH 7"6, containing o.I M-NaC1, o'oo5 M-CaClz, 37 °C, o to 6o rain). 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 o.I.O. Nonidet P4o. 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, I[IO strength GMA I, or 0"05 M-tris/HCl, pH 7"5) with 20 #l O'I mM-potassium iodide. 25 #1 0.25 mM hydrogen peroxide (H202) and 25 td carrier free 125I, Amersham Radiochemical Centre, U.K.; 5o #g intact or disrupted virus. Depending on the ratio of a25I to virus (/~Ci a25I:/~g SYVV = o.ooi to 0"33), different amounts of 12sI were employed. Three 25/~1 samples of 0"25 mm-H202 were added to the reaction at two-minute intervals after a gentle agitation and the addition of 25 #l lactoperoxidase solution (E2so—0.08). When desired, the reaction was stopped by adding 0.5 ml of m-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. I rotor). The pellet was then resuspended in o.I ml o.oi.

RESULTS

After the purified virus was electrophoresed on5,7,5, and IO 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 I, trace (a). According to Wagner et al.'s proposal (I972), the structural proteins' nomenclature has been adopted. The structural proteins' estimated weights, as determined by IO acrylamide gel calculations, were I5OOOO (high molecular weight protein), 83ooo (G), 6o0oo (N), 44ooo (MI), and 36ooo (M2). The minor high weight protein content differed depending on the preparation.

The I5OOOO molecular weight component and G protein had covalently attached carbohydrates, as determined by periodic acid-Schiff's staining (Fig. I, 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, I972). 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 I o 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 I and 2) decreased quickly (by about 5 to 7o0o), 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. (I972) proposal is followed in the nomenclature of the SYVV proteins (Fig. I); however, this is based on location and function as criterion. The nomenclature used here may need to change when additional information becomes available because to the difficulty in definitively determining the location and function of the SYVV proteins, particularly MI and M2. For the structural proteins in this investigation, the tool weight values are comparable to those reported by Schultz & Harrap (I976), with the exception of G protein, which they discovered to be 7r ooo. 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, I975). 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, 969; Knudson & MacLeod, I972). 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, i973; Emerson & Yu, I975;

The Journal of Virology

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 P4o treatment. 0970 Sokol et al. noted Upon SDS/z mercaptoethanol disruption of the aH-glucosamine-labelled virus, an I6OOOO 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, I974). 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.

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The Journal of Virology

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