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The Glycoprotein of Vesicular Stomatitis Virus Is the Antigen That Gives Rise to and Reacts with Neutralizing Antibody

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The glycoprotein, but no other virion protein, of vesicular stomatitis virus was solubilized by the nonionic detergent Triton X-100 in low ionic strength buffer. The solubilized viral glycoprotein induced the synthesis of antibody that formed a single precipitin line with the glycoprotein and that neutralized the infectivity of the virus. The neutralizing activity of the antibody was efficiently blocked by purified glycoprotein.

The virion of vesicular stomatitis (VS) virus is composed of five polypeptide chains (10, 12) in addition to ribonucleic acid and cell-derived lipids and glycolipids (6, 8). One of the polypeptides is a glycoprotein (the G protein) which contains carbohydrate chains (1, 9) and appears to comprise the spikes which protrude from the virion envelope (2, 3, 7). The glycoprotein can be removed selectively from the surface of the virion by proteolytic enzymes, resulting in loss of infectivity, apparently due to degradation of the virion glycoprotein (3, 7). Various detergents also solubilize the glycoprotein of VS virus (2, 3, 11) as well as the glycoprotein of the related rabies rhabdovirus (4). The glycoprotein appears to be the major antigenic determinant and is probably responsible for neutralization of infectivity by antibody and for type specificity of the virus (2, 5).

In this report, we describe a simple method for extracting intact glycoprotein of VS virus essentially uncontaminated with other viral proteins. In addition, we present evidence that the virion glycoprotein is the major immunogen of VS virus.

As previously described (12), the Indiana serotype of VS virus was grown in and plaqued on monolayer cultures of L cells cultivated in basal medium (Eagle) supplemented with 2% inactivated fetal calf serum. In most experiments, stocks of VS virions had been labeled by growing them in the presence of D-[6-^3H]glucosamine (3.6 Ci/mmole) or a ^{14}C-amino acid mixture (57 mCi/matom of carbon), or both, in serum-free basal medium diluted 1:50 with buffered salts. Released virions were collected in medium harvested 18 hr after infection and clarified by centrifugation at 600 × *g* for 10 min. The virions were concentrated and partially purified by two cycles of differential centrifugation at 78,000 × *g* for 90 min followed by rate zonal centrifugation for 90 min at 30,000 × *g* in a 0 to 40% linear sucrose gradient (8). The purified B virions of VS virus were collected from the visible band of infectious virus, pelleted at 78,000 × *g* for 90 min, suspended in 0.5 ml of 0.01 M HEPES buffer (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, *p*H 7.8), disaggregated by ultrasonic vibration, and stored at −70 C.

Although rhabdovirus glycoproteins have been successfully liberated by exposure to nonionic detergents, these preparations are generally contaminated with the other viral envelope protein M (2, 4, 11). The following method was used to solubilize the glycoprotein of VS virions without detectable contamination by other labeled proteins. Triton X-100 was added to purified, labeled VS virions, at a concentration of 4 × 10^8 to 8 × 10^8 plaque-forming units (PFU) per ml of 0.01 M HEPES buffer, *p*H 7.8, to provide a final Triton concentration of 2%. After stirring for 60 min at room temperature, the Triton-virus mixture was centrifuged at 140,000 × *g* for 90 min at 5 C. The proteins of the resultant pellet were suspended in 0.01 M HEPES buffer, extracted by boiling for 2 min in sodium dodecyl sulfate (SDS), and analyzed by electrophoresis on 7.5% polyacrylamide gels (12). Figure 1 reveals that Triton X-100 solubilized virtually all the glycoprotein of VS virions labeled with ^3H-glucosamine and ^{14}C-amino acids (Fig. 1B), whereas the 140,000 × *g* pellet contained all the other virion proteins: L, N, NS, and M (Fig. 1A). These and similar data indicate that the glycoprotein can be obtained relatively free from other virion proteins by this procedure.

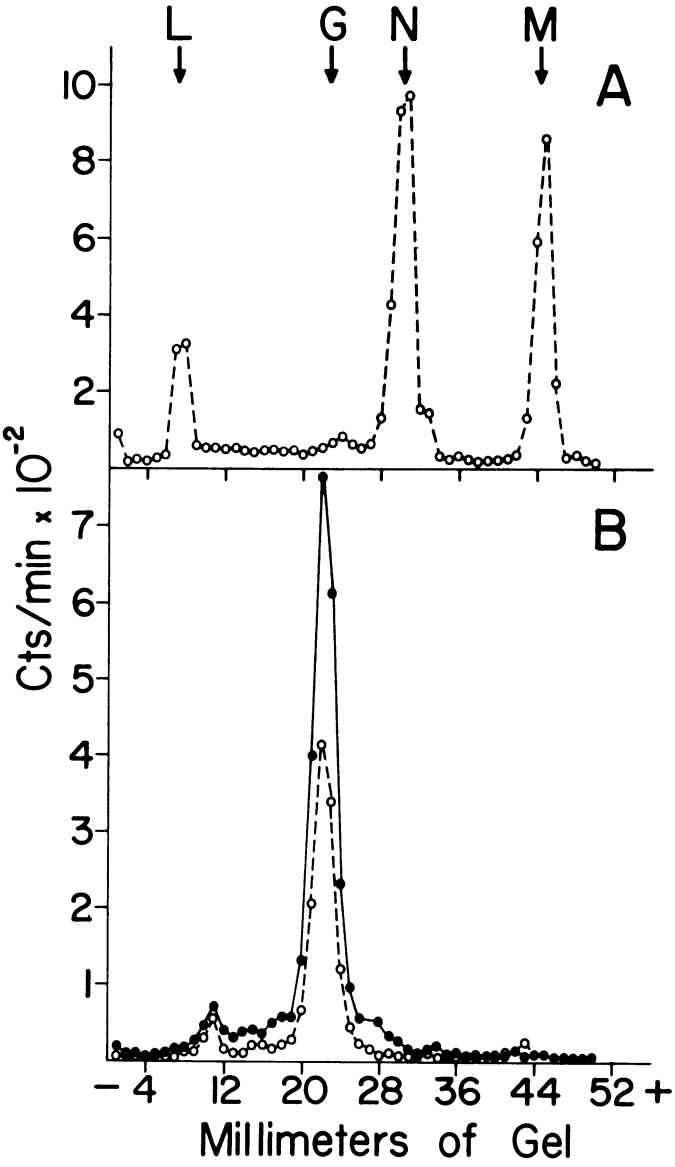

FIG. 1. *Electropherograms of (A) sedimentable and (B) nonsedimentable proteins of VS-B virions treated with Trition X-100. VS virus grown in L cells was labeled with ^{3}H-glucosamine (2 μCi/ml) plus ^{14}C-amino acids (0.5 μCi/ml) for 17 hr. Released virions were purified by differential centrifugation and rate zonal centrifugation in 0 to 40% linear sucrose gradients. About 4 × 10^{8} B virions suspended in 1 ml of 0.01 M HEPES buffer, pH 7.8, were treated with 2% Trition X-100 for 1 hr at room temperature. The virions were then centrifuged at 140,000 × g for 90 min in an SW50L rotor; the supernatant fluid was withdrawn, and the pellet was resuspended in 1 ml of HEPES buffer. The pellet and the supernatant fractions each were made 1% with respect to SDS and 2-mercaptoethanol and were boiled for 2 min, and 0.25 ml was electrophoresed for 5.5 hr on SDS-containing 7.5% polyacrylamide gels at 5 ma gel. The gels were sliced into 1.25-mm lengths, dissolved in a Nuclear-Chicago solubilizer, and counted in a scintillation spectrometer assisted by a double-label computer program. The arrows indicate approximate positions of marker VS viral proteins L, G, N, and M as determined on parallel gels. Protein NS (10, 12) is not marked but should be just to the right of protein N. Symbols: ●——●, ^{3}H-glucosamine (not plotted in panel A, mostly background); ○-----○, ^{14}C-amino acids.*

Antiserum was raised in rabbits immunized by multiple intradermal injections of either Triton-solubilized, electrophoretically homogeneous glycoprotein or purified whole virus, each antigen being suspended in complete Freund adjuvant; boosters were given 5 weeks later by intravenous injection of homologous antigen, and the rabbits were bled the following week. Both of these antisera were tested by two-dimensional immunodiffusion in agar against electrophoretically homogeneous VS viral glycoprotein as well as purified, whole VS virus disrupted with 2% sodium deoxycholate and 2% Triton X-100. Figure 2 illustrates precipitin lines of identity for the reaction of the anti-glycoprotein serum with purified glycoprotein and a detergent-solubilized component of the VS virion; the pelleted cores of detergent-treated virus were free from detectable glycoprotein antigen. Figure 2 also shows that an identical precipitin band was present after diffusion of anti-whole VS viral serum against detergent-solubilized viral glycoprotein and whole, disrupted virus. However, the reaction of whole virus and of pelleted virus cores showed at least one additional antigenic component, presumably the nucleoprotein core antigen described by Cartwright et al. (2, 3).

Figure 3 demonstrates that the anti-glycoprotein serum had a high titer of antibody that neu-

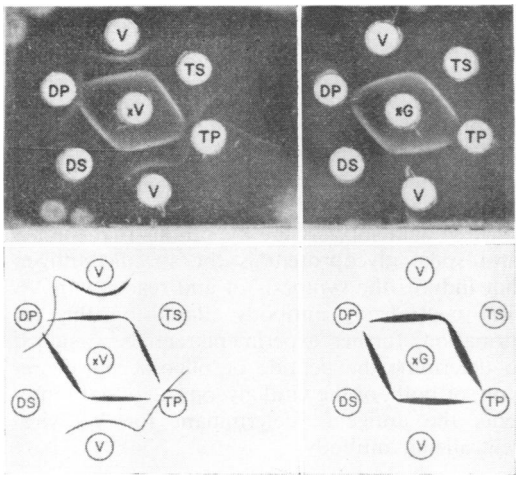

FIG. 2. *Antigen comparison of detergent-fractionated components of VS virus and of whole virus as determined by immunodiffusion against rabbit anti-whole virus serum (xV, left half of figure) and rabbit anti-glycoprotein serum (xG, right half of figure). Purified VS-B virions in 0.01 M HEPES buffer were fractionated by selective solubilization of proteins with either 2% Triton X-100 or 2% Triton X-100 mixed with 2% sodium deoxycholate. After incubation with the detergents at room temperature for 45 min, the virus suspensions were centrifuged at 125,000 × g for 90 min to yield pellet and supernatant fractions containing different mixtures of viral proteins. The fractionated viral proteins or detergent-disrupted unfractionated virus were placed in wells cut in 1% Ionagar (Colab, Inc., Greenwood, Ill.) containing 0.1% sodium deoxycholate buffered with barbital at pH 8.2, and allowed to diffuse against anti-whole virus serum or antiglycoprotein for 48 hr at room temperature in a humidified atmosphere. Proteins in each fraction were identified by SDS-polyacrylamide gel electrophoresis. V, Complete, detergent-disrupted virus (proteins L, G, N, and M); TS, Triton supernatant fraction (G protein); TP, Triton pellet (proteins L, N, and M); DS, Triton plus deoxycholate supernatant fraction (proteins L, N, and M); DP, Triton-deoxycholate pellet (proteins L, N, and M).*

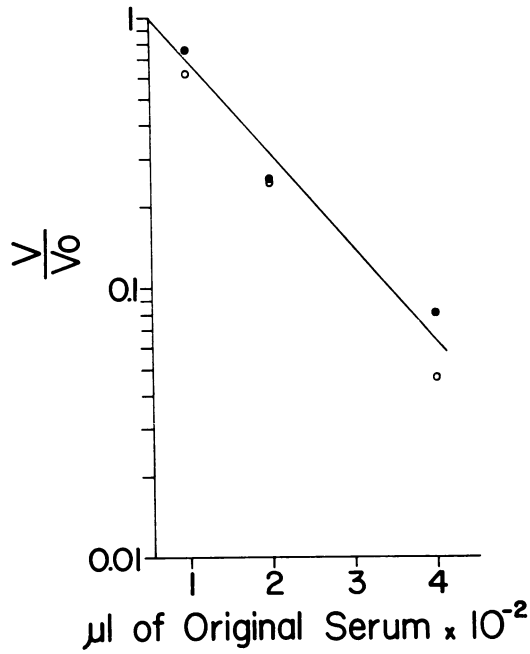

FIG. 3. *Plaque neutralization of VS virus by rabbit antiglycoprotein serum. Antibody produced by inoculation of a rabbit with purified VS viral glycoprotein was diluted such that the final dilution in the reaction mixtures was 10^{-5}, 2.5 × 10^{-5}, or 5 × 10^{-5} (lower dilutions of antiserum completely neutralized the virus). Each dilution of antiserum was mixed for 1 hr at room temperature with two dilutions of VS virus calculated to contain 100 (●) or 1,000 (○) PFU/0.2 ml of plating mixture. Controls consisted of equal amounts of virus mixed with diluent. Duplicate monolayer cultures of L cells were plated with 0.2 ml of each reaction mixture, adsorbed for 1 hr at 37 C, overlaid with medium containing 1% agar, and stained with neutral red after incubation for 48 hr at 37 C. The results are recorded as the fractional reduction of plaques after incubation of virus in antiserum (V) divided by the number of control plaques (V_0) and plotted against microliters of antiserum.*

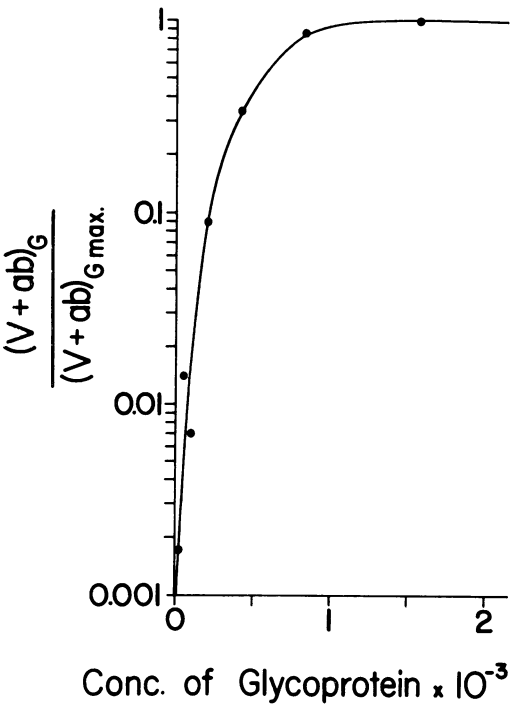

FIG. 4. *Quantitative analysis of the capacity of electrophoretically homogeneous VS viral glycoprotein to reverse the virus-neutralizing activity of anti-glycoprotein serum. Preparation of Triton-released glycoprotein and extraction with acetone are described in the text and preceding figure legends as is the production of glycoprotein antibody. The glycoprotein solution was diluted in twofold steps from 1:320 to 1:40,960, and 0.5 ml of each glycoprotein dilution was added to 0.4 ml of anti-glycoprotein serum diluted in medium to 5 × 10^{-3}, equivalent to 97% plaque-neutralizing activity. After incubation at room temperature for 60 min, 0.1 ml of 3 × 10^7 PFU of VS virus was added to each glycoprotein-antiserum mixture and incubated for an additional 30 min at room temperature. Controls consisted of VS virus in diluent alone, antiserum alone, or glycoprotein alone. The reaction mixtures were plated in duplicate on monolayer cultures of L cells, and the plaques were scored 48 hr after incubation at 37 C. Glycoprotein alone had no effect on viral infectivity at the doses used. The results are recorded as the fractional increase of plaques at each dilution of glycoprotein divided by the glycoprotein concentration that completely reverses antibody-neutralizing activity (restoration of 97-100% of the test PFU added) and plotted against increasing concentrations of glycoprotein.*

tralized the infectivity of Indiana serotype VS virus, although this antiserum had a negligible effect on the plaquing efficiency of the New Jersey serotype of VS virus (data not shown). Comparison of this virus neutralization activity with that of antibody directed against whole VS virus showed almost identical reactivity. We conclude from this experiment that specific anti-glycoprotein serum contains neutralizing potential equivalent to that of anti-whole virus serum.

To obtain additional evidence that the viral glycoprotein antibody is in fact the neutralizing antibody, the solubilized, electrophoretically homogeneous glycoprotein depicted in Fig. 1B was tested for its capacity to block the neutralizing activity of anti-glycoprotein serum. For this purpose, the Triton was removed from the glycoprotein supernatant fraction by a series of three extractions with acetone at −70 C followed by removal of acetone by lyophilization. The extracted glycoprotein was reconstituted in 0.01 M HEPES buffer, diluted serially, and mixed with a constant 5 × 10^{-3} dilution of the anti-glycoprotein serum which was capable of neutralizing 97% of VS viral infectivity. Figure 4 shows that the purified viral glycoprotein, even at relatively high dilutions, effectively reversed the viral neutralizing activity of the monospecific antibody directed against the VS viral glycoprotein.

These data support the hypothesis that the VS viral spike glycoprotein is the specific antigen that induces the synthesis of and reacts with VS viral neutralizing antibody. Based on this information, further experiments can be designed to determine the peptide or oligosaccharide region, or both, of the viral glycoprotein that represents the antigenic determinant for VS viral neutralizing antibody.

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